U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-285 607

Preliminary Sampling and Analytical Procedures for Evaluating the Disposal of Dredged Materials

(U.S.) Environmental Protection Agency, San Francisco, Calif

17 Apr 74

PB 285 607

PRELIMINARY SAMPLING AND ANALYTICAL PROCEDURES FOR EVALUATING THE DISPOSAL OF DREDGED MATERIALS

Laboratory Support Branch
Environmental Protection Agency
Region IX
April, 1974

Environmental
Protection Agency
Region 9
1975

Environmental
Prot Agency
9
75
L ARY

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

154

BIBLIOGRAPHIC DATA SHEET

1. Report No.

2.

3. Recipient's Accession No. PB285607

4. Title and Subtitle
PRELIMINARY SAMPLING AND ANALYTICAL PROCEDURES FOR EVALUATING THE DISPOSAL OF DREDGED MATERIALS

5. Report Date
APRIL 1974

6.

7. Author(s)
HO L. YOUNG, DAVID MINARD, HAROLD SCOTTEN ET AL.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
US ENVIRONMENTAL PROTECTION AGENCY REGION IX
215 FREMONT ST. SAN FRANCISCO CA 94105
LABORATORY SUPPORT BRANCH

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Address
SAME AS box 9.

13. Type of Report & Period Covered

14. 900/00

15. Supplementary Notes

16. Abstracts

THIS MANUAL DESCRIBES METHODS FOR THE ANALYSIS OF DREDGED MATERIALS AND RECEIVING WATERS. ITS PURPOSE IS TO ESTABLISH UNIFORM ANALYTICAL PROCEDURES IN ACCORDANCE WITH THE EPA REGION IX DREDGE SPOIL DISPOSAL CRITERIA.

17. Key Words and Document Analysis. 17a. Descriptors

ANALYTICAL TECHNIQUES
DREDGING
BOTTOM SAMPLING
WATER ANALYSIS

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group 1

18. Availability Statement

19. Security Class (This Report) UNCLASSIFIED

20. Security Class (This Page) UNCLASSIFIED

21. No. of Pages 154

22. Price PCA08 MFA01

11759

PRELIMINARY SAMPLING AND ANALYTICAL PROCEDURES FOR EVALUATING THE DISPOSAL OF DREDGED MATERIALS

Laboratory Support Branch
Environmental Protection Agency
Region IX
April, 1974

1a

PREFACE

This manual describes methods for the analysis of dredged materials and receiving waters. Its purpose is to establish uniform analytical procedures in accordance with the EPA Region IX Dredge Spoil Disposal Criteria.

Most of the methods were taken directly or with slight modification from the following sources:

1. Methods for Chemical Analysis of Water and Wastes, Analytical Quality Control Lab, EPA, 1971.
2. Chemistry Laboratory Manual, Bottom Sediments, Great Lakes, Cincinnati, Ohio 45202.
3. Provisional Methods from the Analytical Quality Control Lab, Cincinnati, Ohio 45202.
4. Federal Register 38, No. 75, Part II. National Pollutant Discharge Elimination System, Appendix A.

The other methods were developed and are being used by the Laboratory Support Branch, EPA, Region IX.

This preliminary manual of selected analytical procedures is still subject to review and subsequent modification. This manual was prepared and assembled by Dr. Ho L. Young, Chief Chemistry Section, David Minard, Harold Scotten, Gary Thompson and Mario Conti.

April 17, 1974

Peter K. Mueller, Ph.D.
Chief, Laboratory Support
Branch

TABLE OF CONTENTS

I. Sampling and Analysis Requirements for Dredge Spoil Disposal 1
  A. Number and Location of Core Samples to be Taken 1
  B. Number of Analyses per Core 1
  C. Analyses to be Performed 2

II. Core Processing 4
  A. Core Preservation 4
  B. Preparation for Analysis 4

III. Standard Elutriate (Shake and Settle Test) 6
  A. Definition 6
  B. Shake Test 6

IV. Analysis of the Mixture of Sediment and Disposal Site Water 12
  A. Immediate Oxygen Demand 12

V. Analyses of Supernatant and Disposal Site Water 15
  A. Suspended Solids (total, prior to Filtration) 15

VI. Analyses of Standard Elutriate and Disposal Site Water 17
  A. Biochemical Oxygen Demand 17
  B. Nitrate Nitrogen 19
  C. Total Kjeldahl Nitrogen (Manual Method) 22
  D. Total Kjeldahl Nitrogen 27
  E. Total Phosphate (Manual Method) 36
  F. Total Phosphate (Automated Method) 40
  G. Method for Chlorinated Phenoxy Acid Herbicides in the Elutriate Test 49
  H. Method for Organohalogen Biocides the Elutriate Test 63
  I. Method for Polychlorinated Biphenyls [PCB'S] in the Elutriate Test 83
  J. Method for Organochlorine Solvents in the Elutriate Test 123

VII. Analyses of Sediment 131
  A. Dry Solids 131
  B. Metals in Bottom Sediments by Atomic Absorption 133
  C. Mercury (Cold Vapor Technique) 137
  D. Oil and Grease 146

I. SAMPLING AND ANALYSIS REQUIREMENTS FOR DREDGE SPOIL DISPOSAL

A. Number and Location of Core Samples to be Taken

For all projects, the number and location of core samples to be taken must be approved by Regional Office personnel on a case-by-case basis. This approval will normally be obtained after a meeting of the applicant or his representatives with the Regional Office personnel.

As a general rule, the following formula will be used to determine the number of cores to be obtained:

$$\text{Number of cores to be obtained} = \frac{\sqrt{A}}{50}, \text{ where } A = \text{area to be dredged (sq. yards)}$$

Ideally, each core should be located so as to be representative of equal sediment volumes. Nearness to pollution sources, irregularity of area to be dredged, and past sediment data may increase or decrease the number of core samples required. All applicants, regardless of project size, will be required to obtain a minimum of one core sample.

B. Number of Analyses per Core

Each core to be analyzed shall be taken from the surface to project depth. It shall then be divided into 3-foot segments, beginning with the surface segment. After division into 3-foot segments, if the remainder at the bottom is 1 1/2 feet or greater, it will be considered as an additional segment. If the remainder is less than 1 1/2 feet, it may be combined with the last 3-foot segment.

C. Analyses to be Performed

1. Substantially Sand

For those materials which the applicant believes are substantially sand, only size distribution data are required. The number of core samples to be collected and analyzed for particle size distribution shall be determined by the following formula:

$$\text{Number of cores} = \frac{\sqrt{A}}{50}, \text{ where } A = \text{area to be dredged (sq. yards)}$$

One analysis shall be performed for each core using a homogenized sample taken from surface to project depth. In the event that the material is not found to be substantially sand, the analyses indicated below are required.

2. 100 Fathom (or Deeper) Ocean Disposal

The following bottom sediment analyses (dry weight basis are required:

Parameter

1. Mercury
2. Cadmium
3. Lead
4. Zinc
5. Oil and grease

3. Inland and Ocean Disposal (less than 100 Fathoms)

The following bottom sediment analyses (dry weight basis) are required:

Parameter

1. Mercury
2. Cadmium
3. Lead
4. Zinc
5. Oil and grease

In addition, the following test on the elutriate and on the water from the disposal site are required:

1. I.O.D. (elutriate only)
2. B.O.D.
3. Suspended Solids
4. Organohalogens

When disposal is proposed for inland waters, the following additional tests on the elutriate and the water from the disposal site are required for projects greater than 50,000 cubic yards:

Parameter

5. Phosphorus (total
6. Total Kjeldahl Nitrogen
7. Nitrate

4. Additional Tests

Additional testing on a case-by-case basis may be required (bioassays, additional major constituents, additional heavy metals).

II. CORE PROCESSING

A. Core Preservation

1. Liners containing cores should be sealed at both ends with vapor proof caps.
2. Cores should be refrigerated to retard bacterial or enzymatic decomposition.
3. Analysis should be started as soon as possible and preferably not later than 48 hours after collection. If a longer delay is unavoidable, cores should be frozen at the time of collection.
4. Cores should be transported in a refrigerated or frozen state by a messenger or by registered mail from the collection area to the laboratory.

B. Preparation for Analysis

1. Thaw frozen samples overnight at room temperature. Each sample container should be placed in a container to prevent loss and contamination of sample.
2. Each core segment should be carefully removed from the liner, by means of a piston-like rod if necessary, into a suitable container such as a glass beaker or tray.
3. The core sample should be passed through a stainless steel sieve (U.S. Series equivalent #10). Larger organisms that might be crushed and forced through the sieve should be removed prior to sieving.

4. If the entire sample is too large to be used for analyses, it may be spread out in a glass tray and random portions selected for sieving. All of the sieved sample should be homogenized and the homogenate used for all analyses except the IOD.

III. STANDARD ELUTRIATE (SHAKE AND SETTLE TEST)

A. DEFINITION

"Standard elutriate" is the supernatant resulting from the vigorous 30-minute shaking of one part of bottom sediment (by weight) in four parts of water from the disposal site (by volume), and allowing to settle for one hour.

B. SHAKE TEST

1. Scope and Application

1.1 This test may be used for assessing the contribution of soluble and partially soluble constituents of dredge materials to receiving water.

1.2 This test will measure:

(a) The dissolved materials in the interstitial waters of the dredge material.

(b) The readily soluble fractions of the solid phases of the dredge material.

(c) The constituents loosely absorbed on the solid phases of the dredge material.

1.3 This test will not measure any of the insoluble or tightly adsorbed material associated with rapidly settleable dredge material.

2. Summary of Test

2.1 A weighed portion of well mixed, wet dredge material (grams) is added to a volume (ml) of receiving water (proposed disposal site water) at a ratio of 1:4, of dredge material:receiving water (W/V). Final volume of the mixture should be at least one liter. The mixture is shaken for thirty minutes under controlled conditions and immediately transferred to a one-liter Erlenmeyer flask to the liter mark and allowed to settle for one hour. The "supernatant" is siphoned to about 5 mm above the clearly defined layer of sediment. The supernatant will be used for the suspended solids determinations. The "Elutriate" is prepared by centrifugation of the supernatant and of the mixture of receiving water and sediment, at moderate speed; then by filtration through 0.45 micron membrane filter. Should more elutriate material be needed the remaining mixture of receiving water and sediment may be centrifuged and filtered also. The elutriate will be used for determining biological oxygen demand, organohalogens, phosphorus, total kjeldahl nitrogen and nitrate analyses. The concentrations of constituents in the supernatant and "elutriate" from the dredge mix are compared with concentrations found in the filtered receiving water.

3. Sample Collection and Preservation

3.1 Collect composite receiving water samples in plastic bottles and immediately store at 4°C.

3.2 Collect dredge material samples in plastic jars or bags ("Whirl-Pak", plastic freezer containers, etc.) and store at 4°C (or in frozen condition if sample is to be held more than 72 hours).

3.3 For all trace organic measurements, water and dredge material samples must be stored in glass containers (see item 5.2).

4. Definitions

4.1 Receiving water - water collected at the proposed disposal site.

4.2 Dredge material - bottom material taken by dredge from one location and transported to another location.

4.3 Supernatant- the aqueous portion of the mixture of receiving water and dredge sediment after it has been shaken and settled.

4.4 Elutriate - the clear fluid of the supernatant after centrifugation and filtration.

5. Apparatus

5.1 Laboratory shaker, Eberbach 6000 with a 6050 Utility Box, or equivalent, capable of shaking a 1 gallon container at 100 excursions/minute.

5.2 Glass jars, wide mouth, one gallon capacity with Teflon lined, screw top lids. Note: It may be necessary to purchase jars and Teflon sheets separately, in which case the Teflon lid liners may be prepared by the laboratory personnel from Teflon sheets. Suggested Source of jars and lids:

Cincinnati Container Corporation
2833 Spring Grove Avenue
Cincinnati, Ohio 45225

Jars, Cat. #120-400-F-0-0-4 (128 oz.)
Lids, Cat. #120-400-White, FTK, PPE

Suggested source of Teflon sheet:

Hughes R. S. Co., Inc.
2818 - 8th Street
Berkeley, CA

Designated Mech. Grade, .020 in. thick.

6. Procedure

6.1 Collect a minimum volume of two gallons of receiving water. If it is known in advance that a large number of measurements are to be performed on the dredge mix sample, a 5-gallon sample may be advisable. Store immediately at 4°C.

6.2 Filter an appropriate portion of the composite receiving water sample through a 0.45 micron membrane filter that has been prerinsed with about 100 ml of the same water.

6.3 Analyze this filtered sample as quickly as possible for the constituents of interest.

6.4 Collect dredge material by coring device. Immediately cool to 4°C or the frozen state until used.

6.5 Seive dredge material through U. S. Standard #10 stainless steel seive.

6.6 Mix the seived dredge material and unfiltered composite disposal site water at a 1:4 ratio of dredge material: disposal site water (W/V), (at room temperature, 22°C ± 2°). Total volume of the mixture should be at least one liter.

6.7 Cap tightly and shake vigorously on an automatic shaker at about 100 excursions per minute for 30 minutes.

6.8 After shaking, the mixture is immediately transferred to a one-liter Erlenmeyer flask(s) to the liter mark and allowed to settle for one hour. The supernatant layer is carefully removed by siphoning to approximately 5 mm above the sediment layer without disturbing the sediment. The supernatant is used for the determination of suspended solids and for preparation of the "Elutriate".

6.9 Centrifuge the supernatant and the remaining receiving water - dredge sediment mixture at 3000 G for 15 minutes.

6.10 Filter the clear liquid through a 0.45 micron membrane filter to obtain a particulate-free solution (the standard elutriate). Store in a clean glass container at 4°C.

6.11 Analyze for: (1) Biochemical oxygen demand (5 day, 20°C), organohalogens, total phosphorus, total kjeldahl nitrogen and nitrate.

7. Criteria

7.1 If SE>1.5 W, the sediment is considered to be polluted, where:

SE = Concentration of a specific constituent in the standard elutriate.

W = Concentration of the same constituent in filtered composite water sample from proposed disposal site.

IV. ANALYSIS OF THE MIXTURE OF SEDIMENT AND DISPOSAL SITE WATER

This analysis measures the change of dissolved oxygen in the receiving water produced by the disposed dredged sediment.

A. IMMEDIATE OXYGEN DEMAND

1. Scope and Application

The test can be conducted immediately in the field if a suitable oxygen probe is available. Otherwise the sample should be preserved with reagents prescribed by the Winkler-Azide analysis for dissolved oxygen, kept in darkness and the analyses completed in a laboratory.

2. Oxygen Probe Procedure

2.1 Weigh 15 gm of wet sediment on a weighing paper or weighing dish. Prepare three such samples. Rinse each sample into separate BOD bottles. The sediment can be cleaned from the weighing container by rinsing with a small amount of disposal site water into the 300 ml BOD bottles.

2.2 Fill the BOD sample bottles and one control bottle with water from the proposed disposal site. To avoid trapping bubbles, fill the BOD bottles from the bottom with a siphon action.

2.3 Measure dissolved oxygen in control bottle (without sediment).

2.4 Stopper and shake bottles containing sediment for one minute. Let stand for 15 minutes.

2.5 After standing fifteen minutes remove stopper and measure dissolved oxygen with a probe.

2.6 Calculation

2.6.1 Wet basis

$$\text{I.O.D., mg/l/g} = \frac{(D_0 - D_1) \times 0.3 \times 1000}{\text{gm sample}}$$

D_0 = mg of dissolved oxygen present in 1 ml of proposed disposal site water (average value of three bottles).

D_1 = mg of dissolved oxygen present in 1 ml of sediment - disposal site water mixture, at 15 minutes after preparation (average value of three bottles).

2.6.2 Dry basis

$$\text{I.O.D. mg/l/g} = \frac{\text{mg/l I.O.D. (Wet Basis)}}{\text{decimal fraction of dry solid}}$$

3. Winkler-Azide Procedure

3.1 Weigh 15 gm of wet sediment on a weighing paper or weighing dish. Prepare three samples. The sediment can be cleaned from the weighing container by rinsing with a small amount of disposal site water into the 300 ml BOD bottles.

3.2 Fill the BOD sample bottles and one control bottle with water from the proposed disposal site. To avoid trapping bubbles, fill the BOD bottles from the bottom with a siphon action.

3.3 Titrate dissolved oxygen in control bottle (without sediment).

3.4 Stopper and shake bottles containing sediment for one minute.

3.5 After fifteen minutes remove stoppers and titrate dissolved oxygen by Winkler-Azide procedure as described in Methods for Chemical Analysis of Water and Wastes, EPA, 1971.

3.6 Calculation:

Same as oxygen probe procedure.

4. Reference:

4.1 Method for Chemical Analysis of Water and Wastes pg. 284, EPA 16020, 1971.

4.2 Standard Methods for the Examination of Water and Wastewater, 13th edition, American Public Health Association, Washington, D.C., 1971.

V. ANALYSES OF SUPERNATANT AND DISPOSAL SITE WATER

A. SUSPENDED SOLIDS (TOTAL, PRIOR TO FILTRATION)

1. Scope and Application

This method is applicable to saline water and supernatant of "shake and settle test". The practical range of the determination is from 20 mg/l to 20,000 mg/l.

2. Summary of Method

Filter 100 ml of well-mixed supernatant from II through a 4.7 cm prewashed glass-fiber filter. The residue retained on the filter is dried to a constant weight at 103° - 105°C.

3. Interferences

High salt content in sea water will produce inaccurately high value.

4. Procedure

Follow that described in the Methods for Chemical Analysis of Water and Wastes, 1971, p. 278, Environmental Protection Agency, Analytical Quality Control Laboratory, Cincinnati, Ohio with the following exceptions:

2.1 Use a 4.7 cm prewashed glass fiber filter

2.2 Immediately after filtration, while vacuum is applied, rinse the filter with three successive 10 ml volumes of glass-distilled water.

5. Calculation

$$\text{TSS, mg/l} = \frac{W_2 - W_1}{V} \times 1000$$

W_2 = Weight of dry filter after filtration

W_1 = Weight of dry filter before filtration

V = Volume of supernatant in ml

VI. ANALYSES OF STANDARD ELUTRIATE AND DISPOSAL SITE WATER

A. BIOCHEMICAL OXYGEN DEMAND

1. Scope and Application

The biochemical oxygen demand test is an empirical bioassay procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at at 20°C for a specified time period (often 5 days).

2. Summary of Method

Each sample of filtered disposal site water and of standard elutriate, or their appropriate dilution, is incubated for 5 days at 20°C in the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.

3. Reagent

3.1 Seeding: Use a septic activator such as "Cultured Plumber" by Gerald Bower, "Septonic" by Fielding Co., or an equivalent. Prepare sufficient seed for the test according to the manufacturers direction. The seed should be acclimated to a high salt condition if the receiving water is sea water. To acclimate, the seed is incubated at 20°C for two days in water containing (a) one ml each of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions for each liter of water (see Standard Methods reference for concentrations); (b) 150 mg/l each of glucose and glutamic acid as carbon source; (c) sodium chloride at a concentration similar to sea water.

3.2 Use 2 ml of the preincubated seed for each liter of incubation medium of the BOD test.

4. Procedures

The procedure to be used is described in Standard Methods for the Examination of Water and Waste Water, 13th Edition, P. 489, Method 219. American Public Health Association, Washington, D.C. 1971.

B. NITRATE NITROGEN

1. Scope and Application

1.1 This method is applicable to the analysis of marine water.

1.2 This method measures 0.05 - 45 ug N/liter.

2. Summary of the Method

2.1 The method is based on the reduction of nitrate to nitrite and the measurement of nitrite. Nitrite analysis must be accomplished at the same time to differentiate between the nitrate nitrogen and the nitrite nitrogen. The method of choice is that found in A Practical Handbook of Seawater Analysis (Ref. 1)

3. Sample Handling and Preservation

3.1 All glassware used in analysis should be cleaned and rinsed with distilled water.

3.2 Adequate information on the effectiveness of preservatives is lacking.

3.3 Samples should be analyzed immediately after collection. If analysis is delayed, preserve the sample with mercuric chloride in order that the final concentration of mercuric chloride is 40 mg/l. The use of mercuric chloride is recommended because of its effectiveness as a biological activity inhibitor (Ref. 2-3).

3.4 Preservation can also be accomplished by freezing and storing the frozen sample in a deep freeze at -20°C (Ref. 1).

4. Interferences

4.1 Waters with high sulfide concentration will interfere because of reaction of the sulfide with cadmium.

4.2 Suspended material will plug up the reductant column; thus, it would be appropriate to filter waters that appear turbid.

5. Apparatus

5.1 The details on the reducing column are presented in II. 6, "The Determination of Reactive Nitrate," pp. 71-76 found in A Practical Handbook of Seawater Analysis (Ref. 1).

6. Reagents

6.1 Reagent grade chemicals free of nitrate are to be used in preparation. Follow the details for preparation of reagents on pgs. 72-76 of A Practical Handbook of Seawater Analysis (Ref. 1).

7. Procedure

7.1 Follow the details of the procedure on pgs. 73-76 in A Practical Handbook of Seawater Analysis (Ref. 1).

8. Calculation

8.1 Report nitrate nitrogen concentration of elutriate in ug/l.

8.2 Obtain a standard curve by plotting the absorbance of standards run by the procedure against NO_3-N, ug/l. Compute concentration of samples by comparing sample absorbance with standard curve.

9. Precision and Accuracy

9.1 Strickland and Parsons report precision levels on page 71 of A Practical Handbook of Seawater Analysis (Ref. 1).

REFERENCES

1. Strickland, J. D. H. and Parson, T. R. II 6 "Determination of Nitrate nitrogen." A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Ottawa, Canada, Bull. 167 pp. 71-76 1968.
2. Weber, C. I. The Preservation of Plankton Grab Samples. Water Pollution Surveillance System Applications and Development Report No. 26, FWPCA, Dept. of Interior, June, 1967.
3. Jenkins, D. A study of methods suitable for the analysis and preservation of nitrogen forms in an estuarine environment. Report to the U.S.P.H.S., Region IX, WSPC Division, SERL No. 65-13, College of Engineering and School of Public Health, University of California, August, 1965.

C. TOTAL KJELDAHL NITROGEN (MANUAL METHOD)

1. Scope and Application

1.1 The Kjeldahl nitrogen is designed as the summation of ammonia nitrogen and those forms of nitrogen which under the conditions of a vigorous catalyzed sulfuric acid reflux are converted to ammonia. The digestion step is usually followed by distillation into an acidified aqueous solution.

1.2 The manual method applicable to marine waters is that found in Methods for Chemical Analysis of Water and Wastes, (Ref. 1).

1.3 Use of macro, semi-micro and micro systems which do not change the chemistry of the procedure is considered to be equivalent.

2. Summary of the Method

2.1 The sample is heated in the presence of sulfuric acid, potassium sulfate and catalyst ($HgSO_4$, SeO_2, $CuSO_4$ or combinations of all) to drive off waters. After the water has been evaporated, the digestion is continued until all organic matter is converted to carbon dioxide and expelled. When the reflux mixture becomes colorless or pale yellow and sulfur dioxide fumes are visible, the reflux action is continued for another thirty minutes to assure complete digestion.

2.2. The digested sulfuric acid mixture is cooled. The neutralization step will also require cooling unless this is accomplished in a closed system with release of vapors into the acidified distillation receiver.

3. Sample Handling and Preservation

3.1 As in the analysis of waters and wastewaters, the question of preservation is unresolved. The best approach is analysis of samples immediately after collection. A widely used preservation technique is freezing of samples in polyethylene containers. The logistics required in collection and transport make freezing an attractive preservation technique.

3.2 Mercuric chloride has also been recommended as an inhibitor of biological activity (Refs. 3-4).

4. Interferences

4.1 No information is available on interference.

5. Apparatus

5.1 A digestion apparatus with adequate venting systems to assist in evaporation of water and to trap chlorine and sulfur dioxide fumes.

5.2 A distillation system that will permit neutralization of the acid solution with the distillate which is received submerged in a weak acid receiver to quantitatively entrap all ammonia vapor.

5.3 Spectrophotometer or filter photometer for use at 640 nm and providing a light path of 10 cm. A shorter light path can be used as values approach 100 ug/kg.

5.4 All the glassware used in sample preparation must be thoroughly cleaned, washed with warm dilute hydrochloric acid and thoroughly rinsed in ammonia-free distilled water. The cleaning of glassware and analysis must be performed in a well ventilated, smoke-free laboratory (smoke from tobacco has been shown to provide a positive interference).

6. Reagents

6.1 The sulfuric acid must be nitrogen-free. Each batch of sulfuric acid must be compared for ammonia blanks. Those batches that provide high ammonia blanks are to be discarded.

6.2 The distilled water used in preparation of reagents, blanks and standards must be free of ammonia. It may be necessary to percolate distilled water through an ion exchange resin specifically formulated to remove ammonia.

6.3 All reagents used in preparation of solutions must be of the highest quality obtainable and free of ammonia.

6.4 The ammonium chloride used to prepare standards must be of reagent grade or better quality.

7. Procedure

7.1 Follow procedure in Methods for Chemical Analysis of Water and Wastes, 1971 (Ref. 1).

7.2 From 7.2 on page 152 of Ref. 1, "Dilute the sample, if required to 500 ml, and add 100 ml sulfuric acid - mercuric sulfate - potassium sulfate solution (Note 2), and evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow." When the solution turns pale yellow and the SO_3 fumes are produced, continue heating for another half an hour. Cool the residue.

7.3 In section 7.7 on page 153 of Ref. 1, determine the ammonia in distillate by using the procedure for ammonia nitrogen analysis.

8. Calculation

8.1 Report Kjeldahl nitrogen concentration of the elutriate in mg/l.

$$\text{TKN mg/l} = \frac{(A-B) \times N \times F \times 1000}{S}$$

A = ml of standard 0.020 N H_2SO_4 solution used in titrating the sample.

B = ml of standard 0.020 N H_2SO_4 solution used in titrating blank.

N = Normality of sulfuric acid solution.

F = Milliequivalent weight of nitrogen (14 mg).

S = Milliliters of sample digested.

9. Precision and Accuracy

9.1 No data is available on precision and accuracy in seawater.

REFERENCES

1. Anonymous. Methods for Chemical Analysis of Water and Wastes. Environmental Protection Agency, NERC, ACQL, Cincinnati, Ohio. 1971.

2. Solorzano, L. "Determination of Ammonia in Natural Waters by the Phenol-Hypochlorite Method." Limnology and Oceanography, Vol. 14, pp. 799-801. 1959.

3. Weber, C.I. The preservation of Plankton Grab Samples. Water Pollution Surveillance System Applications and Development Report No. 26, FWPCA, Dept. of the Interior, June 1967.

4. Jenkins, D. A study of methods suitable for the analysis and preservation of nitrogen forms in an estuarine environment. Report to the USPHS, Region IX, WSPC Division, SERL No. 65-13, College of Engineering and School of Public Health, University of California, August 1965.

D. TOTAL KJELDAHL NITROGEN (SEMIAUTOMATED METHOD)

1. Scope and Application

1.1 This method pertains to the determination of Kjeldahl nitrogen present in saline water. Concentrations in the range 0.05 to 5.0 mg/l Kjeldahl nitrogen may be determined.

1.2 The procedure converts nitrogen components of biological origin such as amino acids, proteins, and peptides to ammonia but may not convert the nitrogenous compounds such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines.

2. Definitions

2.1 Total Kjeldahl is defined as the sum of free ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.

2.2 Organic Kjeldahl nitrogen is defined as the difference obtained by substracting the free ammonia value (see ammonia nitrogen method) from the total Kjeldahl nitrogen value.

3. Summary of the Method

3.1 The sample is heated in the presence of concentrated sulfuric acid K_2SO_4 and $HgSO_4$ and evaporated until SO_3 fumes are obtained and is further heated until the solution becomes colorless or pale yellow. The sample is further digested for 30 minutes. The residue is cooled and diluted. The sample is then poured into cups in the sampler tray. The sample is neutralized in the manifold and the ammonia is measured using the alkaline phenol, hypochlorite, sodium nitroprusside system to yield the blue-colored indophenol related dye the color intensity of which is proportional to the inorganic NH_3-N content of the digested sample.

4. Sample Handling and Preservation

4.1 Samples may be preserved by the addition of 40 mg $HgCl_2$ per kg of sample and stored at 4°C or alternatively quick freezing and storing at -20°C.

5. Interferences

5.1 Calcium and magnesium ions are present in concentrations sufficient to cause precipitation problems during the analysis. This problem is eliminated by using the complexing reagent containing potassium sodium tartrate and sodium citrate.

5.2 Any marked variation in acidity or alkalinity among samples should be eliminated, since the intensity of the color used to quantify the concentration is pH dependent.

5.3 Mercury chloride, used as a preservative, gives a negative interference. This is overcome by adding a comparable amount of $HgCl_2$ to the ammonia standards.

6. Apparatus

6.1 A semi-micro digestion apparatus utilizing 100 ml Kjeldahl digestion flasks is required.

6.2 Technicon Industrial AutoAnalyzer II consisting of the following components:

6.2.1 Sampler II or IV

6.2.2 Modified Analytical Cartridge No. 116-D223-01 or the equivalent.

6.2.3 Proportioning Pump III or IV

6.2.4 Industrial AA-II single channel colorimeter equipped with 15 mm x 1.5 mm I.D. tubular flow cells and 630 nm or 650 nm filters.

6.2.5 Recorder.

7. Reagents

7.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised or a mixture of both strongly acidic cation and strongly basic anion exchange resins. Since organic contamination may interfere with this analysis, use of the resin Dowex XE-75 or equivalent which also tends to remove organic impurities is advised. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

7.2 Digestion reagent: Dissolve 134 grams potassium sulfate, K_2SO_4, in 650 ml ammonia-free distilled water and 200 ml concentrated H_2SO_4. Add, with stirring, a solution prepared by dissolving two grams of red mercuric oxide, HgO, in 25 ml 6N H_2SO_4. Dilute the combined solution to 1 liter. Store above 20°C tc prevent crystallization.

7.3 Hengar Boiling Chips, non-selenized.

7.4 Air used for segmenting the stream should be scrubbed (bubbled) through 5N H_2SO_4 prior to its introduction into the system.

7.5 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 grams of phenol (freshly opened reagent grade) in 50 ml of distilled water. Cautiously add, while cooling under tap water, in small increments with agitation, 190 ml of 20 percent NaOH. Dilute to 1 liter with distilled water. Filter through a glass fiber filter before use.

7.6 Sodium hypochlorite solution ("Clorox"): Dilute 200 ml of any good commercially available liquid household bleach having 5.25 percent available chlorine to one liter with distilled water. Check the label for the 5.25 percent formulation. Beware of the super bleach containing approximately six percent available chlorine.

7.7 Complexing reagent: Dissolve 33 grams of potassium sodium tartrate and 24 grams of sodium citrate in 950 ml of distilled water. Adjust the pH of this solution to 5.0 with concentrated sulfuric acid. Dilute to one liter with distilled water. After allowing to settle overnight in a cool place, filter to remove any precipitate. Then add 1/2 ml of Brij-35* solution and store in a stoppered bottle.

*Brij-35 is the registered trademark of Atlas Chemical Industries, Inc. supplied by Technicon Instruments Corporation under part No. T 21-0110.

7.8 Sodium nitroprusside (0.05%): Dissolve 0.5 grams of sodium nitroprusside in 1 liter of ammonia-free water. (This reagent is also known as sodium nitroferricyanide).

7.9 Stock standard ammonium sulfate (1000 mg/l N): Dissolve 4.717 grams of anhydrous ammonium sulfate, $(NH_4)_2SO_4$, dried at 105°C, in ammonia-free water, and dilute to 1 liter. 1 ml = 1.0 mg NH_3-N.

7.10 Standard solution A: Dilute 10.0 ml of stock standard to 1 liter with ammonia-free water. 1 ml = 0.01 mg NH_3-N.

7.11 Using standard solution A, prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

NH_3-N mg/l	ml Std. Solution A/100 ml
0.10	1.0
0.30	3.0
0.50	5.0
1.0	10.0
3.0	30.0
5.0	50.0

8. Procedure

8.1 Digestion

8.1.1 To 50 ml of sample or standard add 10 ml of digestion reagent and a few Hengar boiling chips. Heat the flasks sufficiently to bring the samples to a brisk boil and continue until SO_3 fumes are obtained and the solution turns colorless or pale yellow. Continue the digestion for an additional 30 minutes. The residue is cooled and diluted with 50 ml of distilled water. Standards as well as samples should be digested in this manner.

8.2 For a working range of 0.05 to 5.0 mg/l NH_3-N, set up the manifold as shown in the attached flow chart.

8.3 Allow both colormeter (with 650 nm filters and 15 mm flow cell) and recorder to warm up for 30 minutes. Run a base line with all reagents, feeding ammonia-free water through sample line. Adjust the base line knob and aperture opening on the colorimeter to obtain a proper base line.

8.4 Use a 50 sample/hour, 2:1 sample/wash sampler cam and operate the colorimeter in the Damp 1 mode. All sample cups should be washed with 1 normal hydrochloric acid and rinsed thoroughly with distilled water in order to remove any traces of ammonia. A final rinse should be made with the sample when filling the sampling tray. The sample tray should be covered during the run and the tray filled with sample just prior to loading the tray on the sampler.

8.5 A blank reading for the particular seawater of interest should be determined by sampling the digested seawater while the nitroprusside and hypochlorite reagent lines are in distilled water. The blank obtained should then be subtracted from the readings of the samples with the reagent lines in their proper solutions.

8.6 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

8.7 Switch sample line from distilled water to sampler and begin analysis.

9. Calculations

9.1 Report Kjeldah nitrogen concentration of the elutriate in mg/l.

9.2 Prepare appropriate standard curve derived from processing digested ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

10. Precision and Accuracy

10.1 No data available for saline waters.

REFERENCES

1. Anonymous. Methods for Chemical Analysis of Water and Wastes. Environmental Protection Agency, NERC, AQCL, Cincinnati, Ohio, pgs. 141-147. 1971.

2. APHA, AWWA, WPCF. Standard Methods for Examination of Water and Wastewater, 13th Edition, pgs. 244 -248. 1971.

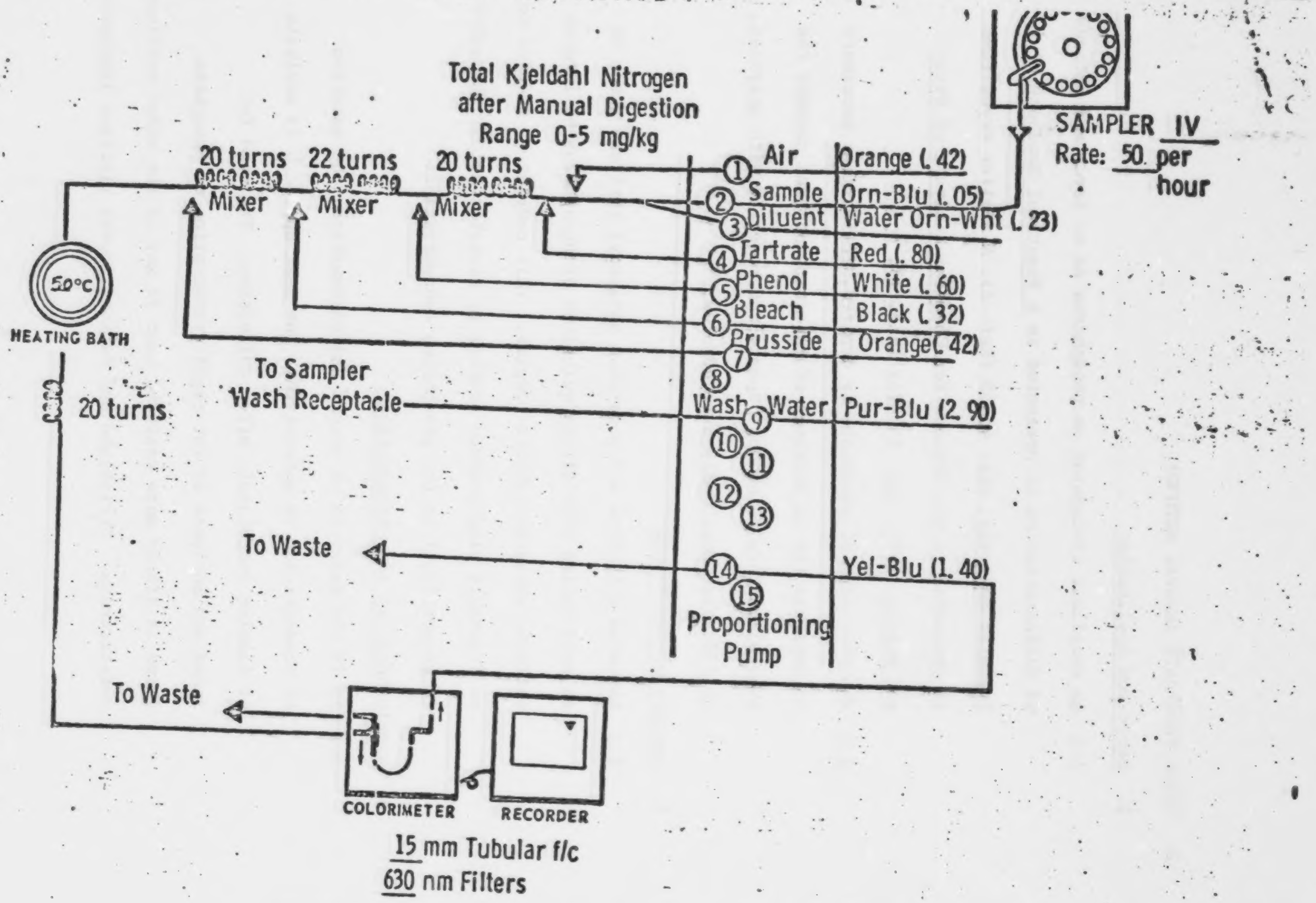

Total Kjeldahl Nitrogen
after Manual Digestion
Range 0-5 mg/kg
SAMPLER IV
Rate: 50 per hour
20 turns
Mixer
22 turns
Mixer
20 turns
Mixer
50°C
HEATING BATH
20 turns
To Sampler Wash Receptacle
To Waste
To Waste
① Air Orange (.42)
② Sample Orn-Blu (.05)
③ Diluent Water Orn-Wht (.23)
④ Tartrate Red (.80)
⑤ Phenol White (.60)
⑥ Bleach Black (.32)
⑦ Prusside Orange (.42)
⑧
⑨ Wash Water Pur-Blu (2.90)
⑩
⑪
⑫
⑬
⑭ Yel-Blu (1.40)
⑮
Proportioning Pump
COLORIMETER
RECORDER
15 mm Tubular f/c
630 nm Filters

FIGURE 1. TOTAL KJELDAHL NITROGEN AFTER MANUAL DIGESTION MANIFOLD

E. TOTAL PHOSPHATE (MANUAL METHOD)

1. Scope and Application

1.1 An excellent discussion on phosphorus as to forms and methods of differentiation is presented in A Practical Handbook of Seawater Analysis, pgs. 45-63 (Ref. 2). A similar exposition is presented in the Methods for Chemical Analysis of Water and Wastes, 1971, pgs. 235-269 (Ref. 1).

1.2 Whatever form of phosphate is desired, it is first necessary to separate the constituent of interest and then convert the phosphorus form to orthophosphate and react it with molybdate to form the phosphomolybdate complex.

2. Summary of the Method

2.1 Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

3. Sample Handling and Preservation

3.1 As in the analysis of waters and wastewaters, the question of preservation is unresolved. The best approach is analysis of samples immediately after collection. The need for immediate analysis or for rapid preservation of phosphate forms is likely more critical than in any of the other nutrient constituents. Strickland and Parsons impose rigorous limitations on the start of analysis or preservation (Ref. 1).

3.2 Quick freezing within 30 minutes after collection in a glycol - carbon dioxide bath at -20°C is recommended by Strickland and Parsons (Ref. 1).

3.3 Mercuric chloride has also been recommended as an inhibitor of biological activity (Ref. 3-4).

4. Interferences

4.1 Silicate, copper and iron do not interfere when present in concentrations normally found in seawater (Ref. 5-6).

4.2 Arsenate interferes on a 1:1 basis. Thus, at normal arsenate and phosphate levels found in seawater the effect of arsenate approaches the error in the phosphate analysis. However, at low phosphate concentrations where specialized procedures may be in use, the arsenate interference is serious. In freshwaters the procedure of D. L. Johnson is used to eliminate arsenate interference (Ref. 7). No studies have been conducted to evaluate the Johnson procedure in marine waters (Ref. 7).

5. Apparatus

5.1 The apparatus sections in Methods for Chemical Analysis of Water and Wastes, pgs. 240-241 and in A Practical Handbook of Seawater Analysis, pgs. 49-62 (Refs. 1-2), provide adequate information on the items which are necessary.

6. Reagents

6.1 Details on reagents are also found in the documents cited in 5.1

7. Procedure

7.1 Follow the procedure found in either of the two methods manuals cited in 5.1.

8. Calculation

8.1 Report the phosphate concentration of elutriate in u/l P.

8.2 Prepare standard curve by plotting absorbance values of standards as ordinates and the corresponding phosphorus concentrations as abscissas.

8.2.1 Process standards and blank exactly as the samples. Run at least a blank and two standards with each series of samples. If the standards do not agree within ± 2% of the true value, prepare a new calibration curve.

8.3 Obtain concentration value of sample directly from prepared standard curve. Report results as P, ug/l.

9. Precision and Accuracy

9.1 Precision information is reported in the study conducted and reported by Hager, et.al. (Ref. 6). Hager's data was converted from uM PO_4 to ug P and is presented below (Ref. 6).

Number of Replicates	Mean Concentration ug/l	Standard Deviation ug/l
6	37.8	.6
5	36.5	.9
11	30.7	.3

REFERENCE

1. Anonymous. Methods for Chemical Analysis of Water and Wastes. Environmental Protection Agency, NERC, ACQL, Cincinnati, Ohio. 1971.

2. Strickland, J.D.H. and Parsons, T.R. II. 9 "Determination of Ammonia." A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa, Canada, pp. 49-64. Bull. 167. 1968.

3. Weber, C.I. The Preservation of Plankton Grab Samples. Water Pollution Surveillance System Applications and Development Report No. 26, FWPCA. Dept. of the Interior. June 1967.

4. Jenkins, D. A study of methods suitable for the analysis and preservation of phosphorus forms in an estuarine environment. Report to the USPHS, Region IX, WSPC Division, SERL No. 65-18, College of Engineering and School of Public Health, University of California. November 1965.

5. Murphy, J. and Riley, J. "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." Anal. Chim Acta. Vol. 27, 31-36. 1962.

6. Hager, S.W., et. al. "A Comparison at Sea of Manual and AutoAnalyzer Analyses of Phosphate, Nitrate and Silicate." Limnology and Oceanography. Vol. 17, pp. 931-937. 1972.

7. Johnson, D.L. "Simultaneous Determination of Arsenate and Phosphate in Natural Waters." Environmental Science and Technology. Vol 5. pp. 411-414. 1971.

F. TOTAL PHOSPHATE (AUTOMATED METHOD)

1. Scope and Application

1.1 An excellent discussion on phosphorus as to forms and methods of differentiation is presented in A Practical Handbook of Seawater Analysis, pgs. 45-63 (Ref. 2). A similar exposition is presented in the Methods for Chemical Analysis of Water and Wastes, 1971, pgs. 235-269 (Ref. 1).

1.2 Whatever form of phosphate is desired, it is first necessary to separate the constituent of interest and then convert the phosphorus form to orthophosphate and react it with molybdate to form the phosphomolybdate complex.

2. Summary of the Method

2.1 Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

2.2 The arsenic reducing reagent is introduced into the analytical scheme before the introduction of the working reagent (Ref. 3).

3. Sample Handling and Preservation

3.1 As in the analysis of waters and wastewaters, the question of preservation is unresolved. The best approach is analysis of samples immediately after collection. The need for immediate analysis or for rapid preservation of phosphate forms is likely more critical than in any of the other nutrient constituents. Strickland and Parsons impose rigorous limitations on the start of analysis or preservation (Ref. 1).

3.2 Quick freezing within 30 minutes after collection in a glycol - carbon dioxide bath at -20°C is recommended by Strickland and Parsons (Ref. 1).

3.3 Mercuric chloride has also been recommended as an inhibitor of biological activity (Ref. 4,5).

4. Interferences

4.1 Silicate, copper and iron do not interfere when present in concentrations normally found in seawater (Refs. 6-7).

4.2 Arsenate interferes on a 1:1 basis. Thus, at normal arsenate and phosphate levels found in seawater the effect of arsenate approaches the error in the phosphate analysis. However, at low phosphate concentrations where specialized procedures may be in use, the arsenate interference is serious. In freshwaters the procedure of D. L. Johnson is used to eliminate arsenate interference (Ref. 3). No studies have been conducted to evaluate the Johnson procedure in marine waters (Ref. 3).

5. Apparatus

5.1 Technicon AutoAnalyzer II (Industrial) consisting of the following components:

5.1.1 Sampler II or IV and Pump IV.

5.1.2 Analytical Cartridge, Technicon part No. 116-D241-01 or equivalent.

5.1.3 Industrial colorimeter, single channel, equipped with 50 mm tubular flow cell and 880 nm filters.

5.1.4 Recorder.

6. Reagents

6.1 Distilled water: Because of possible contamination, this should be prepared by passage through an ion exchange column comprised of a mixture of both strongly acidic-cation and strongly basic-anion exchange resins. The regeneration of the ion exchange column should be carried out according to the manufacturer's instructions.

6.2 All reagents must be of a reagent grade quality or better level and low in phosphate.

6.3 Reagents for automated analysis.

6.3.1 Add 110 ml of concentrated sulfuric acid to 800 ml of deionized, distilled water while cooling. After this solution has been cooled, dilute to one liter with deionized, distilled water.

6.3.2 Dissolve 27 g of ammonium molybdate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

6.3.3 Dissolve 18 g of U.S.P. quality ascorbic acid in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water. The ascorbic acid solution is stable for about two months if kept in a freezer or refrigerator. It is stable for about two weeks if not refrigerated. However, the container must be kept well stoppered.

6.3.4 Dissolve 3.0 g of antimony potassium tartrate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

6.4 Preparation of combined working reagent to 100 ml (110 g) of sulfuric acid (6.2.1) add with stirring 30 ml (30 g) ammonium molybdate solution (6.2.2), 60 ml (60 gm) of ascorbic acid solution (6.2.3) and 10 ml (10 g) of antimony potassium tartrate solution (6.2.4). This mixed reagent must be prepared daily and the unused portion must be discarded.

6.5 Reagents for arsenic interference.

6.5.1 Dissolve 5.6 g of sodium metabisulfite in 25 ml of distilled water and dilute to 40 ml.

6.5.2 Dissolve 0.56 grams sodium thiosulfate in 25 ml of distilled water and dilute to 40 ml.

6.6 While stirring add 20 ml of sulfuric acid (6.3.1) to 40 ml of sodium metabisulfite solution and 40 ml of sodium thiosulfate solution. The mixed reagent is stable for not more than 24 hours if kept refrigerated. The sodium metabisulfite must be prepared fresh just before use. The sodium thiosulfate solution is more stable and can be made up in larger volume.

6.7 Dissolve 5 g of sodium chloride in 800 ml of water and add 2 ml of Levor IV* and make up to one liter. Use this as dilute water.

6.8 Digestant solution for total phosphate digestion and total dissolved phosphate.

6.8.1 Prepare daily by adding 40 g of ammonium persulfate to 250 ml (18.2 g) sulfuric acid (6.3.1).

6.9 Stock phosphate solution. Dissolve 0.4393 g of potassium dihydrogen phosphate in 800 ml of distilled water and dilute to one liter. 1 ml = 0.1 mg P.

6.9.1 Prepare appropriate solution to cover range of analysis of interest.

7. Procedure

7.1 For total phosphate and total dissolved phosphate add 0.5 ml of digestant solution (6.8.1) per 10 ml of sample in a test tube fabricated for screw cap closure. Place screw cap with teflon liner on test tube and tighten snugly. Autoclave the samples at 132°C and place in tray for analysis.

7.7.1 The manifold arrangement is enclosed under the title "Total Phosphate in Seawater after Manual Digestion."

7.7.2 A blank reading for the seawater of interest is run through the manifold while pumping distilled water through the manifold in place of the combined working reagent line.

---

*Levor IV is a wetting agent marketed by Technicon Instrument Corp., under part No. T21-0110.

7.2 For orthophosphate phosphorus use manifold arrangement as indicated in the orthophosphate method. Samples that are turbid should be filtered.

7.2.1 Determine blank correction for the seawater of interest by substituting distilled water for the combined working reagent line and pumping samples through.

8. Calculation

8.1 Standards are run before and after each group of samples (a group will vary from 20 to 120 samples). The concentration over peak height relationship is computed for each set of standards and interpolated over the two runs.

8.2 A salt correction is computed and all values are corrected.

8.3 A saltwater blank is run every 20th sample.

8.4 Report phosphate concentration of elutriate in ug/l.

9. Precision and Accuracy

9.1 Precision and accuracy data is unavailable at this time (7/30/73)

REFERENCES

1. Anonymous. Methods for Chemical Analysis of Water and Wastes. Environmental Protection Agency, NERC, ACQL, Cincinnati, Ohio. 1971.

2. Strickland, J.D.H. and Parsons, T.R. II. 9 "Determination of Ammonia." A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa, Canada. pp. 49-64. Bull. 167. 1968

3. Johnson, D.L. "Simultaneous Determination of Arsenate and Phosphate in Natural Waters." Environmental Science and Technology. Vol. 5 pp. 411-414. 1971.

4. Weber, C.I. The Preservation of Plankton Grab Samples. Water Pollution Surveillance System Applications and Development Report No. 26, FWPCA, Dept. of the Interior. June, 1967.

5. Jenkins, S. A Study of methods suitable for the analysis and preservation of phosphorus forms in an estuarine environment. Report to the USPHS, Region IX, WSPC Division, SERL No. 65-18, College of Engineering and School of Public Health, University of California. November 1965.

6. Murphy, J. and Riley, J. "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." Anal. Chim. Acta.

7. Hager, S.W., et. al. "A Comparison at Sea of Manual and AutoAnalyzer Analyses of Phosphate, Nitrate and Silicate." Limnology and Oceanography. Vol. 17, pp. 931-937. 1972.

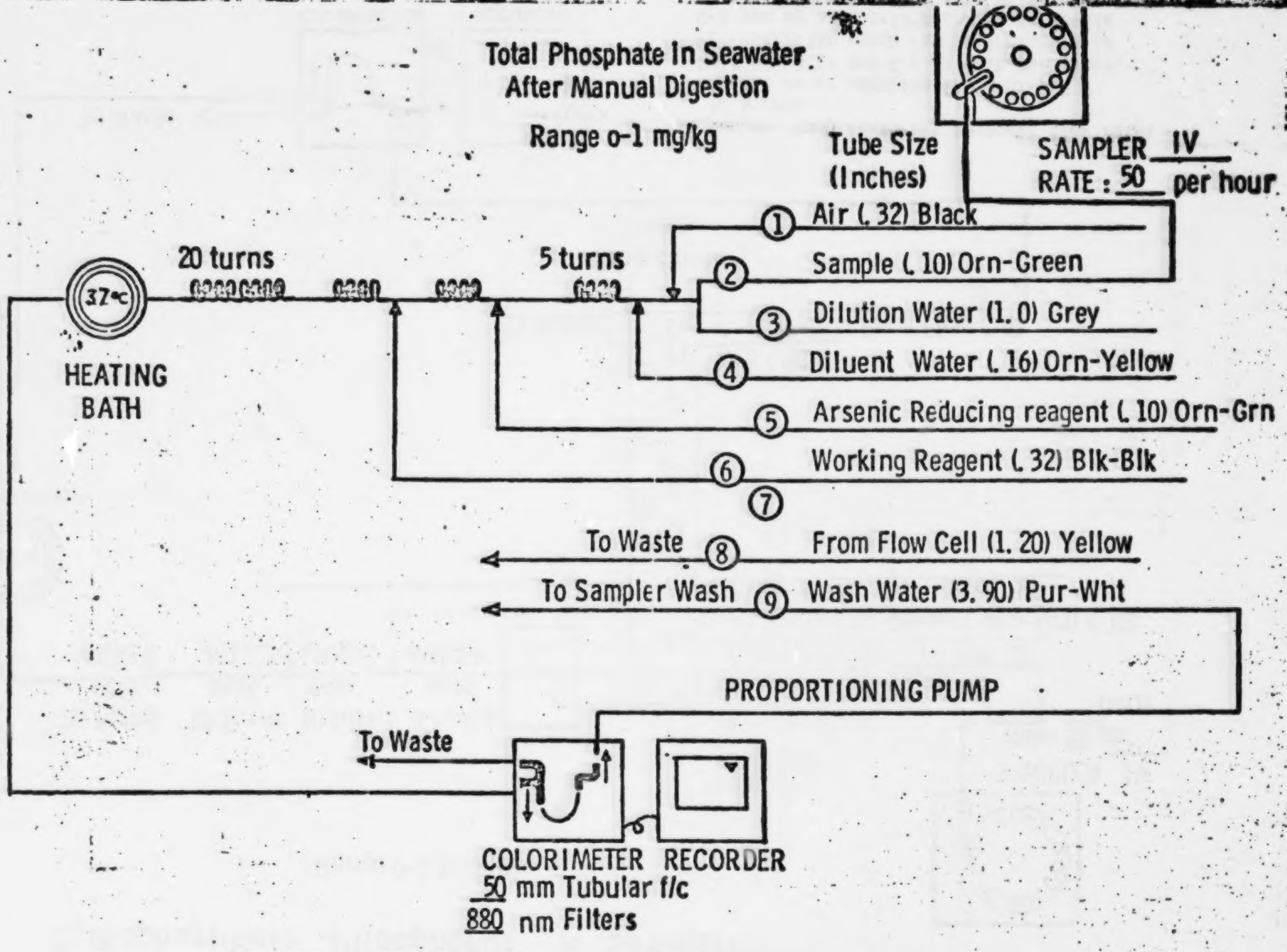
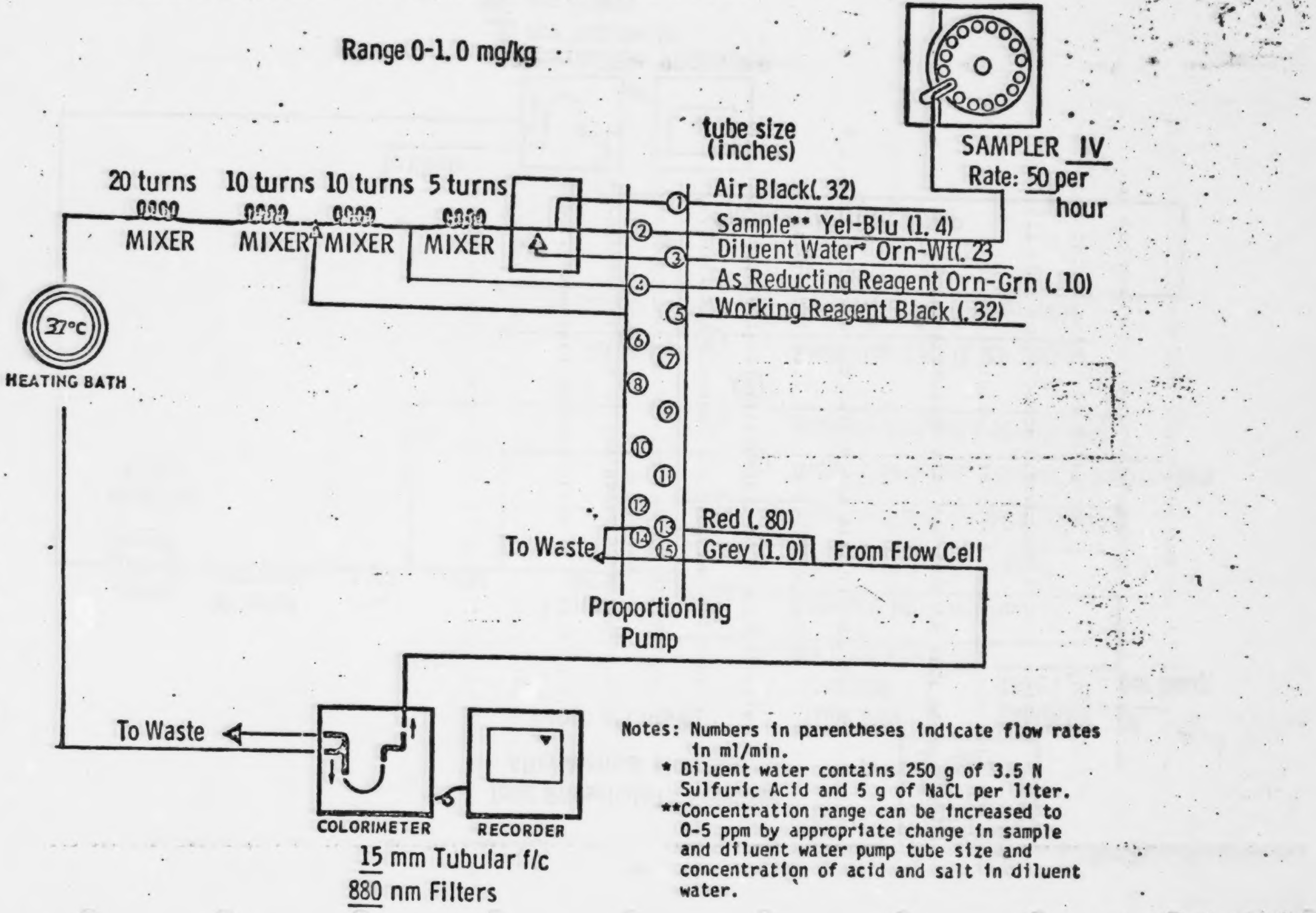

Total Phosphate In Seawater
After Manual Digestion
Range o-1 mg/kg
Tube Size
(Inches)
SAMPLER IV
RATE : 50 per hour
① Air (.32) Black
② Sample (.10) Orn-Green
③ Dilution Water (1.0) Grey
④ Diluent Water (.16) Orn-Yellow
⑤ Arsenic Reducing reagent (.10) Orn-Grn
⑥ Working Reagent (.32) Blk-Blk
⑦
To Waste ⑧ From Flow Cell (1.20) Yellow
To Sampler Wash ⑨ Wash Water (3.90) Pur-Wht
PROPORTIONING PUMP
20 turns
5 turns
37°C
HEATING BATH
To Waste
COLORIMETER
RECORDER
50 mm Tubular f/c
880 nm Filters

FIGURE 5. TOTAL PHOSPHATE IN SEAWATER AFTER MANUAL DIGESTION MANIFOLD

Orthophosphate Phosphorus in Seawater

Range 0-1.0 mg/kg


tube size (inches)
SAMPLER IV
Rate: 50 per hour
20 turns
MIXER
10 turns
MIXER
10 turns
MIXER
5 turns
MIXER
37°C
HEATING BATH
Air Black(.32)
Sample** Yel-Blu (1.4)
Diluent Water* Orn-Wt(.23
As Reducting Reagent Orn-Grn (.10)
Working Reagent Black (.32)
Red (.80)
Grey (1.0)
From Flow Cell
To Waste
Proportioning Pump
To Waste
COLORIMETER
RECORDER
15 mm Tubular f/c
880 nm Filters

Notes: Numbers in parentheses indicate flow rates in ml/min.

* Diluent water contains 250 g of 3.5 N Sulfuric Acid and 5 g of NaCL per liter.

**Concentration range can be increased to 0-5 ppm by appropriate change in sample and diluent water pump tube size and concentration of acid and salt in diluent water.

G. METHOD FOR CHLORINATED PHENOXY ACID HERBICIDES IN THE ELUTRIATE TEST*

1. Scope and Application

1.1 This method covers the determination of chlorinated phenoxy acid herbicides in industrial effluents. The compounds 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), 2,3-dichloro-o-anisic acid (dicamba) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may be determined by this procedure.

1.2 Since these compounds may occur in water in various forms (i.e., acid, salt, ester, etc.) a hydrolysis step is included to permit the determination of the active part of the herbicide. The method may be applied to additional phenoxy acids and certain phenols. However, the analyst must demonstrate the usefulness of the method for each specific compound before applying it to sample analysis.

2. Summary

2.1 Chlorinated phenoxy acids and their esters are extracted from the acidified elutriate with ethyl ether. The esters are hydrolyzed to acids and extraneous organic material is removed by a solvent wash. The acids are converted to methyl esters which are extracted from the aqueous phase. The extract is cleaned up by passing it through a micro-adsorption column. Identification of the esters is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography (1). Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

---

*Taken from "METHOD FOR CHLORINATED PHENOXY ACID HERBICIDES IN INDUSTRIAL EFFLUENTS.", N.P.D.E.S., FED. REG., 38, No. 75, Pt. II. 1973.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part 1, Sections 1.4 and 1.5, (2).

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of chlorinated phenoxy acid herbicides. Sample clean-up procedures are generally required and may result in loss of certain of these herbicides. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Organic acids, especially chlorinated acids, cause the most direct interference with the determination. Phenols including chlorophenols will also interfere with this procedure.

3.4 Alkaline hydrolysis and subsequent extraction eliminates many of the predominant chlorinated insecticides which might otherwise interfere with the test.

3.5 The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Glassware and glass wool should be acid-rinsed and sodium sulfate should be acidified with sulfuric acid to avoid this possibility.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection port.

4.2 Detector Options:

- 4.2.1 Electron Capture - Radioactive (tritium or nickel-63)
- 4.2.2 Microcoulometric Titration
- 4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

- 4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)
- 4.4.2 Glass Wool - Silanized
- 4.4.3 Solid Support - Gas-Chrom-Q (100-120 mesh)
- 4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.
  - 4.4.4.1 OV-210, 5%
  - 4.4.4.2 OV-17, 1.5% plus QF-1, 1.95%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

- 4.5.1 Snyder Column - three ball (macro) and two ball (micro)
- 4.5.2 Evaporative Flasks - 250 ml
- 4.5.3 Receiver Ampuls - 10 ml, graduated
- 4.5.4 Ampul Stoppers

4.6 Blender - High speed, glass or stainless steel cup.

4.7 Graduated cylinders - 100 and 250 ml.

4.8 Erlenmeyer flasks - 125 ml, 250 ml ground glass $\overset{T}{S}$ 24/40

4.9 Microsyringes - 10, 25, 50 and 100 µl.

4.10 Pipets - Pasteur, glass disposable (140 mm long X 5 mm ID).

4.11 Separatory Funnels - 60 ml and 2000 ml with Teflon stopcock.

4.12 Glass wool - Filtering grade, acid washed.

4.13 Diazald Kit - recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. #210,025-2)

4.14 Florisil - PR grade (60-100 mesh) purchased activated at 1250F and stored at 130 C.

5. Reagents, Solvents and Standards

5.1 Boron Trifluoride-Methanol-esterification-reagent, 14 percent boron trifluoride by weight.

5.2 N-methyl-N-nitroso-p-toluenesulfonamide (Diazald) - High purity, melting point range 60-62 C. Precursor for the generation of diazomethane (see Appendix I).

5.3 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.4 Potassium Hydroxide Solution - A 37 percent aqueous solution prepared from reagent grade potassium hydroxide pellets and reagent water.

5.5 Potassium Iodide - (ACS) 10% solution in distilled water.

5.6 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.7 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.8 Sodium Sulfate, Acidified. -- (ACS) granular sodium sulfate, treated as follows: Add 0.1 ml of conc. sulfuric acid to 100 g of sodium sulfate slurried with enough ethyl ether to just cover the solid. Remove the ether with the vacuum. Mix 1 g of the resulting solid with 5 ml of reagent water and ensure the mixture to have a pH below 4. Store at 130 C.

5.9 Sulfuric acid. -- (ACS) concentrated, Sp. Gr. 1.84.

5.9.a. Carbitol (diethylene glycol monoethyl ether).

5.10 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.10.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.10.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.10.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.11 Benzene Hexane - Nanograde, redistilled in glass, if necessary.

5.12 Pesticide Standards - Acids and Methyl Esters, reference grade.

5.12.1 Stock standard solutions - Dissolve 100 mg of each herbicide in 60 ml ethyl ether; then make to 100 ml with redistilled hexane. Solution contains 1 mg/ml.

5.12.2 Working standard - Pipet 1.0 ml of each stock soln into a single 100 ml volumetric flask. Make to volume with a mixture of ethyl ether and hexane (1:1). Solution contains 10 μg/ml of each standard.

5.12.3 Standard for Chromatography - (Diazomethane Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube and evaporate off the solvent using steam bath. Add 2 ml diazomethane to the residue. Let stand 10 minutes with occasional shaking, then allow the solvent to evaporate spontaneously. Dissolve the residue in 200 μl of hexane for gas chromatography.

5.12.4 Standard for Chromatography -(Boron Trifluoride Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube. Add 0.5 ml of Benzene and evaporate to 0.4 ml using a two-ball Snyder microcolumn and a steam bath. Proceed as in 11.3.1. Esters are then ready for gas chromatography.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when ≤ 0.06 ng is injected for electron capture detection and ≤ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards, prepared from methyl esters of phenoxy acid herbicides calculated as the acid equivalent, are injected frequently as a check on the stability of operating conditions.

6.3 The elution order and retention ratios of methyl esters of chlorinated phenoxy acid herbicides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed the use of quality control charts is recommended (3).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample, if suspended matter is present.

8.2 For a sensitivity requirement of 1 μg/1, when using electron capture for detection, take 100 ml of sample for analysis. For microcoulometric or electrolytic conductivity detection, take 1-liter of sample. Background information on the extent and nature of interferences will assist the analyst in selecting the proper sample size and detector.

8.3 Quantitatively transfer the proper aliquot of sample into a two-liter separatory funnel, dilute to one liter and acidify to approximately pH 2 with concentrated sulfuric acid. Check pH with indicator paper.

9. Extraction

9.1 Add 150 ml of ether to the sample in the separatory funnel and shake vigorously for one minute.

9.2 Allow the contents to separate for at least ten minutes. After the layers have separated, drain the water phase into a one-liter Erlenmeyer flask. Then collect the extract in a 250 ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.

9.3 Extract the sample two more times using 50 ml of ether each time, and combine the extracts in the Erlenmeyer flask. (Rinse the one-liter flask with each additional aliquot of extracting solvent.)

10. Hydrolysis

10.1 Add 15 ml of distilled water and a small boiling stone to the flask containing the ether extract, and fit the flask with a 3-ball Snyder column. Evaporate the ether on a steam bath and continue heating for a total of 60 minutes.

10.2 Transfer the concentrate to a 60 ml separatory funnel. Extract the basic solution two times with 20 ml of ether and discard the ether layers. The herbicides remain in the aqueous phase.

10.3 Acidify the contents of the separatory funnel by adding 2 ml of cold (4 C) 25 percent sulfuric acid (5.9). Extract the herbicides once with 20 ml of ether and twice with 10 ml of ether. Collect the extracts in a 125 ml Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate (5.8). Allow the extract to remain in contact with the sodium sulfate for approximately two hours.

11. Esterification (4,5)

11.1 Transfer the ether extract, through a funnel plugged with glass wool, into a Kuderna-Danish flask equipped with a 10 ml graduated ampul. Use liberal washings of ether. Using a glass rod, crush any caked sodium sulfate during the transfer.

11.1.1 If esterification is to be done with diazomethane, evaporate to approximately 4 ml on a steam bath (do not immerse the ampul in water) and proceed as directed in Section 11.2.

11.1.2 If esterification is to be done with boron trifluoride, add 0.5 ml benzene and evaporate to about 5 ml on a steam bath. Remove the ampul from the flask and further concentrate the extract to 0.4 ml using a two-ball Snyder microcolumn and proceed as in 11.3.

11.2 Diazomethane Esterification

11.2.1 Disconnect the ampul from the K-D flask and place in a hood away from steam bath. Adjust volume to 4 ml with ether, add 2 ml diazomethane, and let stand 10 minutes with occasional swirling.

11.2.2 Rinse inside wall of ampul with several hundred microliters of ethyl ether. Take sample to approximately 2 ml to remove excess diazomethane by allowing solvent to evaporate spontaneously (room temperature).

11.2.3 Dissolve residue in 5 ml of hexane. Analyze by gas chromatography.

11.2.4 If further clean-up of the sample is required, proceed as in 11.3.4 substituting hexane for benzene.

11.3 Boron Trifluoride Esterification

11.3.1 After the benzene solution in the ampul has cooled, add 0.5 ml of borontrifluoride-methanol reagent. Use the two-ball Snyder micro column as an air-cooled condenser and hold the contents of the ampul at 50 C for 30 minutes on the steam bath.

11.3.2 Cool and add about 4.5 ml of a neutral 5 percent aqueous sodium sulfate solution so that the benzene-water interface is in the neck of the Kuderna-Danish ampul. Seal the flask with a ground glass stopper and shake vigorously for about one minute. Allow to stand for three minutes for phase separation.

11.3.4 Pipet the solvent layer from the ampul to the top of a small column prepared by plugging a disposable Pasteur pipet with glass wool and packing with 2.0 cm of sodium sulfate over 1.5 cm of Florisil adsorbent. Collect the eluate in a graduated ampul. Complete the transfer by repeatedly rinsing the ampul with small quantities of benzene and passing the rinses through the column until a final volume of 5.0 ml of eluate is obtained. Analyze by gas chromatography.

12. Calculation of Results

12.1 Determine the methyl ester concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2 (2).

(1) $$\text{Micrograms/liter} = \frac{(A)\quad(B)\quad(V_t)}{(V_i)\quad(V_s)}$$

$A = \frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_1= Volume of extract injected (µl)

V_t= Volume of total extract (µl)

V_s= Volume of water extracted (ml)

12.2 Molecular weights for the calculation of the methyl esters as the acid equivalents.

2,4-D	222.0	Dicamba	221.0
2,4-D Methyl ester	236.0	Dicamba methyl ester	236.1
Silvex	269.5	2,4,5-T	255.5
Silvex methyl ester	283.5	2,4,5-T methyl ester	269.5

13. Reporting Results

13.1 Report results in micrograms per liter as the acid equivalent without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

Table 1

RETENTION RATIOS FOR METHYL ESTERS OF SOME CHLORINATED PHENOXY ACID HERBICIDES RELATIVE TO 2,4-D

Liquid Phase[1]	1.5% OV-17 + 2.95% QF-1	5% OV-210
Column Temp.	185 C	185 C
Argon/Methane Carrier Flow	70 ml/min	70 ml/min
Herbicide	RR	RR
2,4-D	1.00	1.00
silvex	1.34	1.22
2,4,5-T	1.72	1.51
dicamba	0.60	0.61
2,4-D (minutes absolute)	2.00	1.62

[1] All columns glass, 180 cm X 4 mm ID, solid support Gas Chrom Q (100/120 mesh)

REFERENCES

(1) Goerlitz, D. G., and Lamar, W. L., "Determination of Phenoxy and Herbicides in Water by Electron-Capture and Microcoulometric Gas Chromatography", U. S. Geol. Survey Water-Supply Paper 1817-C (1967).

(2) "Methods for Organic Pesticides in Water and Wastewater", (1971) U. S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio.

(3) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (1972), U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268.

(4) Metcalf, L. D., and Schmitz, A. A., "The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis", Analytical Chemistry, 33, 363 (1961).

(5) Schlenk, H. and Gellerman, J. L., "Esterification of Fatty Acids with Diazomethane on a Small Scale", Analytical Chemistry, 32, 1412 (1960).

(6) "Pesticide Analytical Manual", U. S. Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.

(7) Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

APPENDIX I

Diazomethane in ether (6).

1. CAUTIONS. Diazomethane is very toxic. It can explode under certain conditions. The following precautions should be observed.

Avoid breathing vapors.

Use only in well-ventilated hood.

Use safety screen.

Do not pipette solution of diazomethane by mouth.

For pouring solutions of diazomethane, use of gloves is optional.

Do not heat solutions to 100 C (EXPLOSIONS).

Store solutions of gas at low temperatures (Freezer compartment of explosion proof refrigerators).

Avoid ground glass apparatus, glass stirrers and sleeve bearings where grinding may occur (EXPLOSIONS).

Keep solutions away from alkali metals (EXPLOSIONS).

Solutions of diazomethane decompose rapidly in presence of solid material such as copper powder, calcium chloride, boiling stones, etc. These solid materials cause solid polymethylene and nitrogen gas to form.

2. PREPARATION.

Use a well-ventilated hood and cork stoppers for all connections. Fit a 125 ml long-neck distilling flask with a dropping funnel and an efficient condenser set downward for distillation. Connect the condenser to two receiving flasks in series - a 500 ml Erlenmeyer followed by a 125 ml Erlenmeyer containing 30 ml ether. The inlet to the 125 ml Erlenmeyer should dip below the ether. Cool both receivers to 0 C.

As water bath for the distilling flask, set up a 2-liter beaker on a stirplate (hot plate and stirrer), maintaining temperature at 70 C.

Dissolve 6 g KOH in 10 ml water in the distilling flask (no heat). Add 35 ml Carbitol (diethylene glycol monoethyl ether), stirring bar, and another 10 ml ether. Connect the distilling flask to the condenser and immerse distilling flask in water bath. By means of the dropping funnel, add a solution of 21.5 g Diazald in 140 ml ether over a period of 20 minutes. After distillation is apparently complete, add another 20 ml ether and continue distilling until distillate is colorless. Combine the contents of the two receivers in a glass bottle (WITHOUT ground glass neck), stopper with cork, and freeze overnight. Decant the diazomethane from the ice crystals into a glass bottle, stopper with cork, and store in freezer until ready for use. The final solution may be stored up to six months without marked deterioration.

The 21.5 g of Diazald reacted in this manner produce about 3 g of Diazomethane.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1151

H. METHOD FOR ORGANOHALOGEN BIOCIDES THE ELUTRIATE TEST*

1. Scope and Application

1.1 This method covers the determination of various organochlorine pesticides, including some pesticidal degradation products and related compounds in industrial effluents. Such compounds are composed of carbon, hydrogen, and chlorine, but may also contain oxygen, sulfur, phosphorus, nitrogen or other halogens.

1.2 The following compounds may be determined individually by this method with a sensitivity of 1 μg/liter: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, pentachloronitrobenzene and trifluralin. Under favorable circumstances, Strobane, toxaphene, chlordane (tech.) and others may also be determined. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

1.3 When organochlorine pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

2. Summary

2.1 The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and/or the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography and liquid-liquid partition, methods for elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography. Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4 and 1.5, (1).

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organochlorine pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organochlorine pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Polychlorinated Biphenyls (PCB's) - Special attention is called to industrial plasticizers and hydraulic fluids such as the PCB's which are a potential source of interference in pesticide analysis. The presence of PCB's is indicated by a large number of partially resolved or unresolved peaks which may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures (2,3).

3.4 Phthalate Esters - These compounds, widely used as plasticizers, respond to the electron capture detector and are a source of interference in the determination of organochlorine pesticides using this detector. Water leaches these materials from plastics, such as polyethylene bottles and tygon tubing. The presence of phthalate esters is implicated in samples that respond to electron capture but not to the microcoulometric or electrolytic conductivity halogen detectors or to the flame photometric detector.

3.5 Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, eg, parathion, also respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. Such compounds can be identified by their response to the flame photometric detector (4).

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection port.

4.2 Detector Options:

- 4.2.1 Electron Capture - Radioactive (tritium or nickel 63)
- 4.2.2 Microcoulometric Titration
- 4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

- 4.4.1 Tubing - Pyrex (180 cm long x 4 mm ID)
- 4.4.2 Glass Wool - Silanized
- 4.4.3 Solid Support - Gas-Chrom Q (100-120 mesh)
- 4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.
  - 4.4.4.1 OV-1, 3%
  - 4.4.4.2 OV-210, 5%
  - 4.4.4.3 OV-17, 1.5% plus QF-1, 1.95%
  - 4.4.4.4 QF-1, 6% plus SE-30, 4%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

- 4.5.1 Snyder Column - three ball (macro) and two ball (micro)
- 4.5.2 Evaporative Flasks - 500 ml
- 4.5.3 Receiver Ampuls - 10 ml, graduated
- 4.5.4 Ampul Stoppers

4.6 Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50 and 100 μl

4.9 Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Graduated cylinders - 100 and 250 ml /

4.12 Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

5. Reagents, Solvents, and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

5.4 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.5 Sodium Sulfate - (ACS) Granular, anhydrous.

5.6 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.7.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.7.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.7.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.8 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - nanograde, redistill in glass if necessary

5.9 Pesticide Standards - Reference grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when $\leq$ 0.06 [illegible] is injected for electron capture detection and $\leq$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.

6.3 The elution order and retention ratios of various organochlorine pesticides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended (5).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For a sensitivity requirement of 1 µg/l, when using microcoulometric or electrolytic conductivity methods for detection take 100 ml of sample for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

8.3 Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Analyze by gas chromatography unless a need for cleanup is indicated. (See Section 10).

10. Clean-up and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides. Of the pesticides listed in Scope (1.2) only mirex is not efficiently recovered.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

10.2.4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition 10.4).

Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

10.3.5 Analyze by gas chromatography.

10.4 Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin
BHC
Chlordane
DDD
DDE
DDT
Heptachlor
Heptachlor Epoxide
Lindane
Methoxychlor
Mirex
Pentachloronitrobenzene
Strobane
Toxaphene
Trifluralin
PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

11. Calculation of Results

11.1 Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2. (1).

(1)

$$\text{Micrograms/liter} = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

$A = \frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (μl)

V_t = Volume of total extract (μl)

V_s = Volume of water extracted (ml)

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

REFERENCES

1. "Method for Organic Pesticides in Water and Wastewater," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268, 1971.

2. Monsanto Methodology for Aroclors - Analysis of Environmental Materials for Biphenyls, Analytical Chemistry Method 71-35, Monsanto Company, St. Louis, Missouri 63166, 1970.

3. "Method for Polychlorinated Biphenyls in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268, 1973.

4. "Method for Organophosphorus Pesticides in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati Ohio 45268, 1973.

5. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

6. "Pesticide Analytical Manual," U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, Washington, D.C.

7. "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Perrine Primate Research Laboratories, Perrine, Florida 33157, 1971.

8. Mills, P.A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).

9. Goerlitz, D.F. and Brown, E., "Methods for Analysis of Organic Substances in Water," Techniques of Water Resources Investigations of the United States Geological Survey, Book 5, Chapter A3, U.S. Department of the Interior, Geological Survey, Washington, D.C. 20402, 1972, pp. 24-40.

10. Steere, N.V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

*Taken from "METHOD FOR ORGANOCHLORINE PESTICIDES IN INDUSTRIAL EFFLUENTS" N.P.D.E.S., FED. REG., 38, No. 75, Pt. II. 1973.

Table 1

RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	1.5% OV-17 + 2.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200 C	180 C	180 C	200 C
Argon/Methane Carrier Flow	60 ml/min	70 ml/min	70 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Trifluralin	0.39	1.11	0.33	0.57
α-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p'-DDT	3.16	2.70	2.69	2.39
p,p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

[1] All columns glass, 180 cm X 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 **A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.**

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

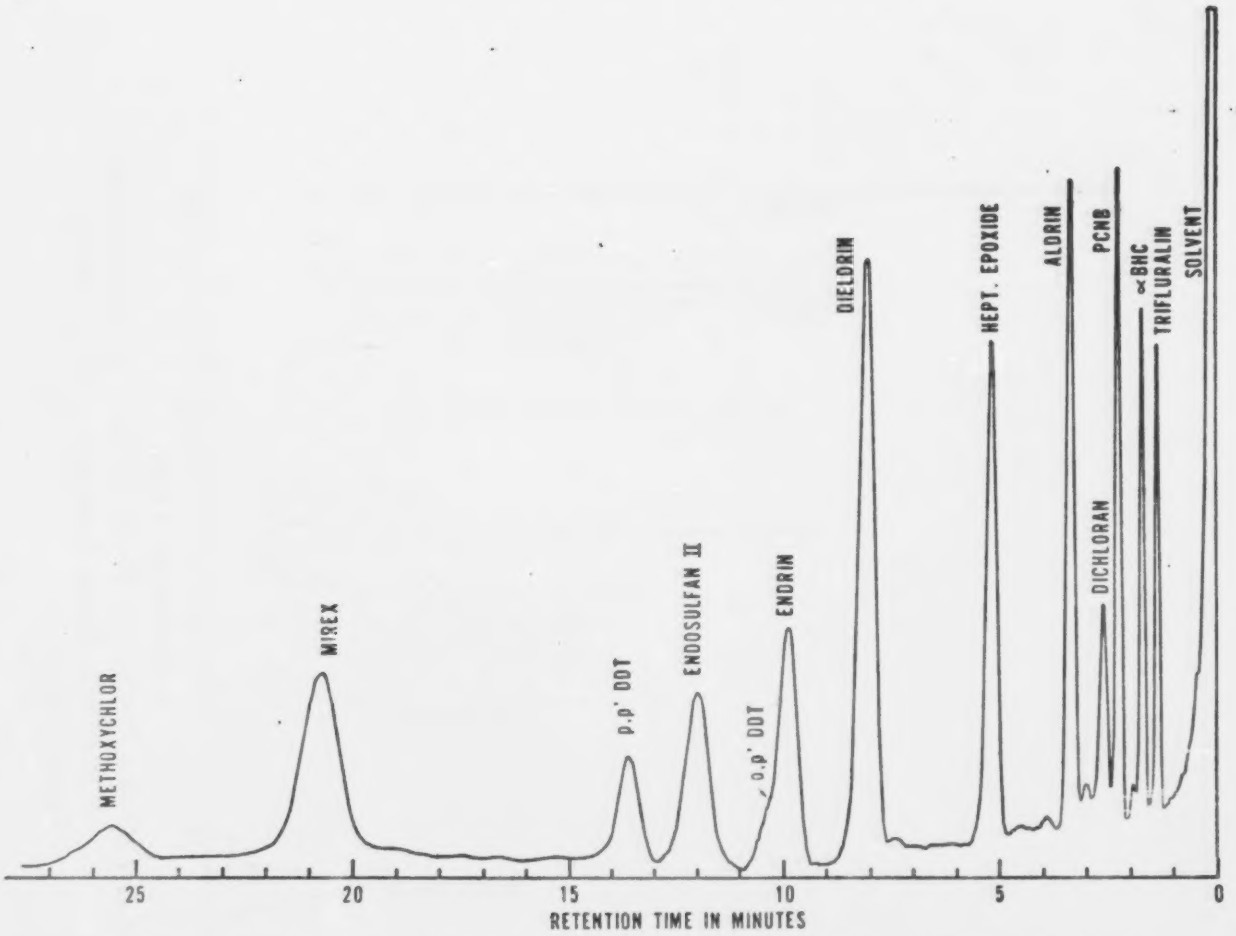


Figure 1. Column Packing: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

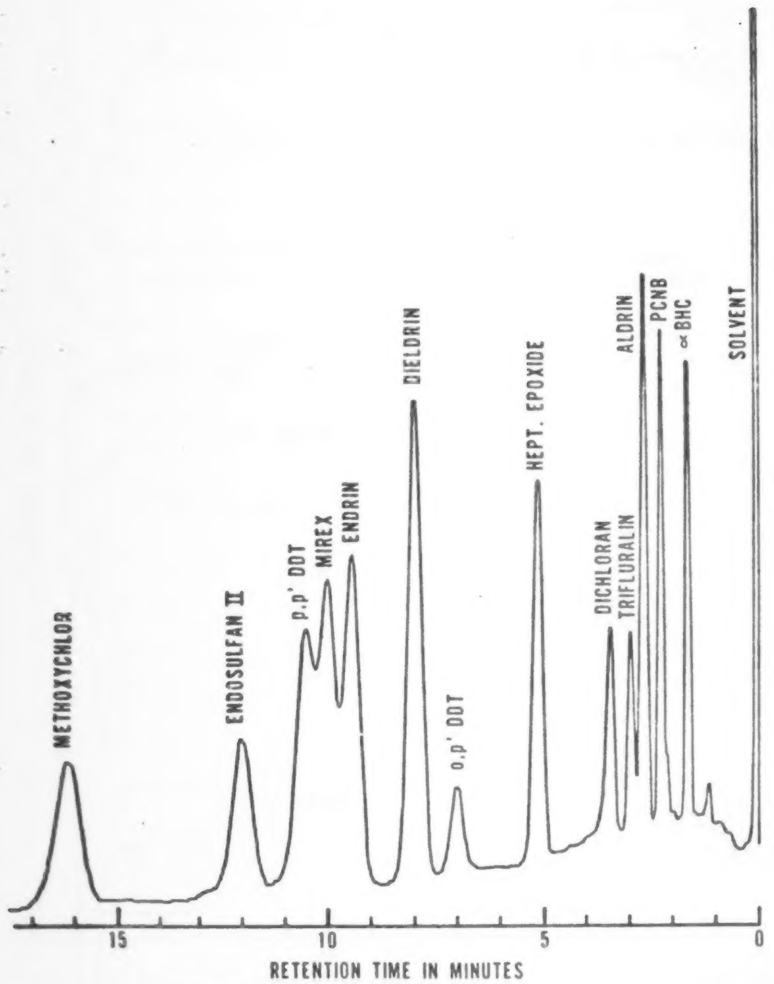


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

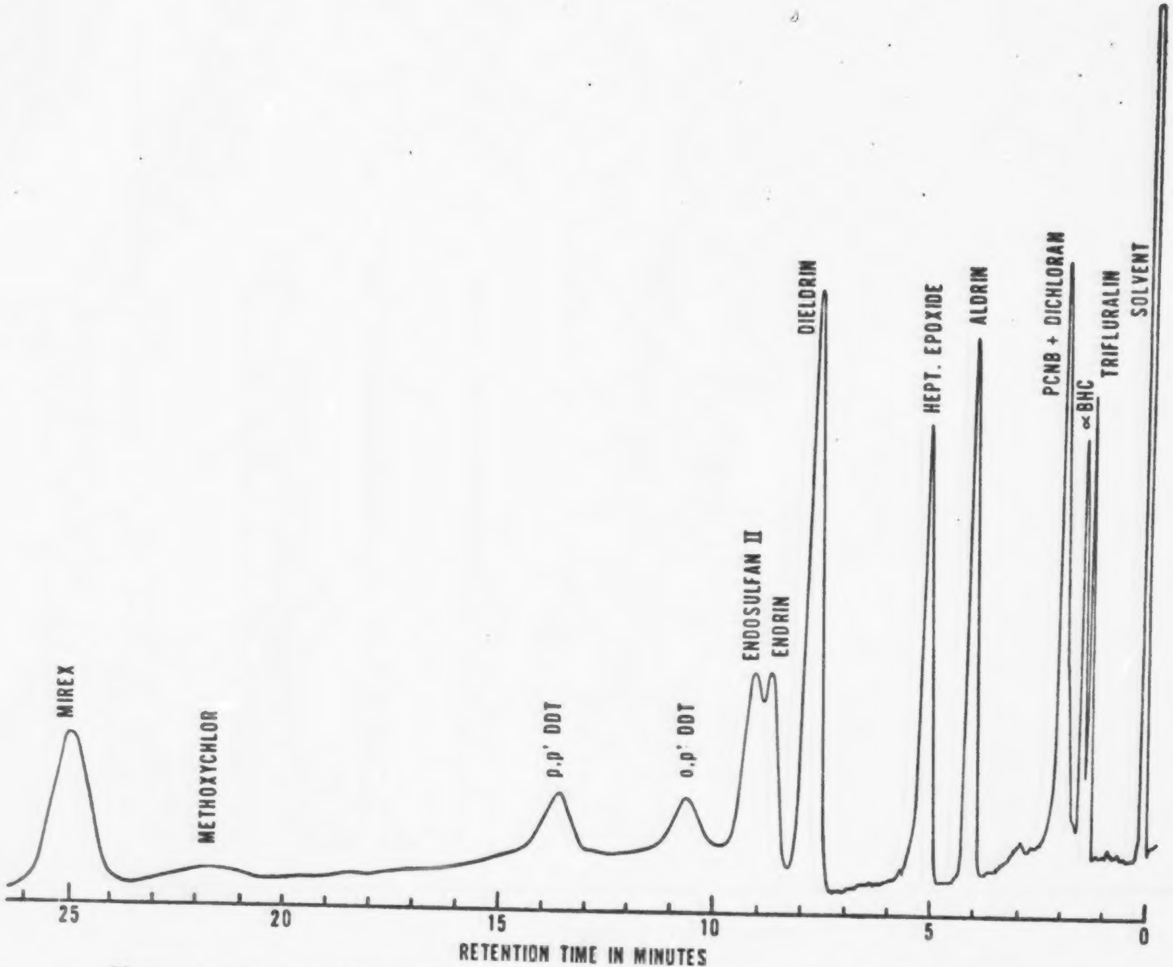


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1145

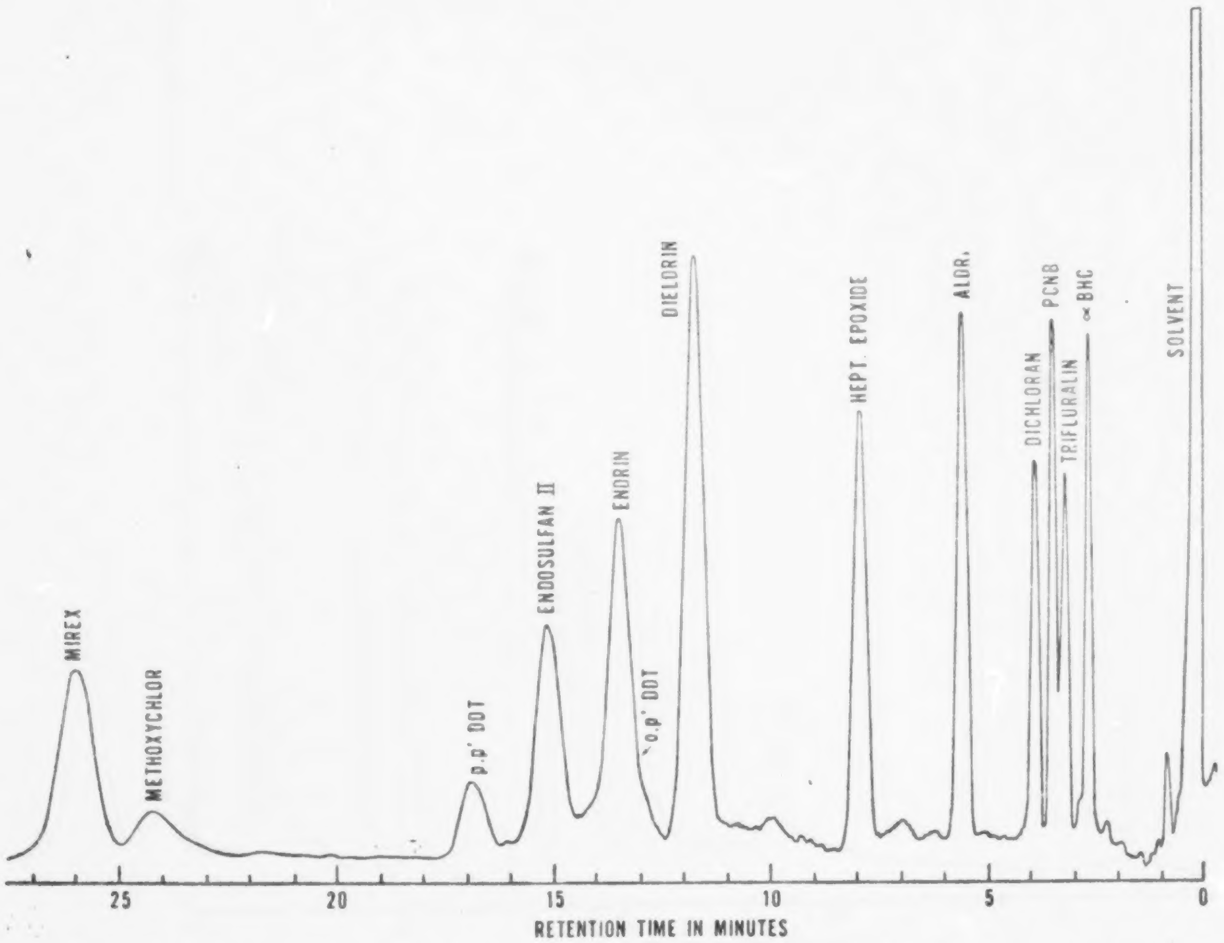


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

I. METHOD FOR POLYCHLORINATED BIPHENYLS [PCB'S] IN THE ELUTRIATE TEST*

1. Scope and Application

1.1 This method covers the determination of certain polychlorinated biphenyl (PCB) mixtures including: Aroclors 1221, 1232, 1242, 1248, 1254, 1260 and 1016.

1.2 The method is an extension of the method for organohalogen biocides in industrial effluents (1). It is designed so that determination of both the PCB's and the organochlorine pesticides may be made on the same sample.

1.3 The limit of detection is approximately 1 ug/l for each Aroclor mixture.

2. Summary

The PCB's and the organochlorine pesticides are co-extracted by liquid-liquid extraction and, insofar as possible, the two classes of compounds separated from one another prior to gas chromatographic determination. A combination of the standard Florisil column cleanup procedure and a silica gel microcolumn separation procedure (2)(3) are employed. Identification is made from gas chromatographic patterns obtained through the use of two or more unlike columns. Detection and measurement is accomplished using an electron capture, microcoulometric, or electrolytic conductivity detector. Techniques for confirming qualitative identification are suggested.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the condition of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to (4), Part I, Sections 1.4 and 1.5.

---

*Taken from "METHOD FOR POLYCHLORINATED BIPHENYLS [PCB'S] IN INDUSTRIAL EFFLUENTS.", N.P.D.E.S., FED. REG., 38, No. 75, Pt. II. 1973.

3.2 The interferences in industrial effluents are high and varied and pose great difficulty in obtaining accurate and precise measurement of PCB's and organochlorine pesticides. Separation and cleanup procedures are generally required to eliminate these interferences; however, such techniques may result in the loss of certain organochlorine compounds. For this reason great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial wastes.

3.3 Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection. These materials do not interfere when the microcoulometric or electrolytic conductivity detectors are used in the halogen mode.

3.4 Organochlorine pesticides and other halogenated compounds constitute interferences in the determination of PCB's. Most of these are separated by the method described below. However, certain compounds, if present in the sample, will occur with the PCB's. Included are: Sulfur, Heptachlor, aldrin, DDE, technical chlordane, mirex, and to some extent o,p'-DDT and p,p'-DDT.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection part.

4.2 Detector Options:

4.2.1 Electron Capture - Radioactive (tritium or nickel-63)

4.2.2 Microcoulometric Titration

4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with detector system.

4.4 **Gas Chromatographic Column Materials:**

- **4.4.1** Tubing - Pyrex (180 cm long X 4 mm ID)
- **4.4.2** Glass Wool - Silanized
- **4.4.3** Solid Support - Gas-Chrom Q (100-120 mesh)
- **4.4.4** Liquid Phases - Expressed as weight percent coated on solid support:
  - 4.4.4.1 SE-30 or OV-1, 3%
  - 4.4.4.2 OV-17, 1.5% + QF-1, 1.95%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

- 4.5.1 Snyder Columns - three ball (macro)
- 4.5.2 Evaporate Flasks - 500 ml
- 4.5.3 Receiver Ampuls - 10 ml, graduated
- 4.5.4 Ampul stoppers

4.6 Chromatographic Column - Chromaflex (400 mm long X 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - Pyrex (approximately 400 mm long X 20 mm ID) with a coarse fritted plate on bottom.

4.8 Micro Column Pyrex - constructed according to Figure 1.

4.9 Capillary pipets disposable (5-3/4 in.) with rubber bulb. (Scientific Products P5205-1).

4.10 Low pressure regulator - 0 to 5 PSIG - with low-flow needle valve (See Figure 1, Matheson Model 70).

4.11 Beaker - 100 ml

4.12 Micro syringes - 10, 25, 50 and 100 µl.

4.13 Separatory Funnels - 125 ml, 1000 ml, and 2000 ml with Teflon stopcocks.

4.14 Graduated Cylinders - 100 ml, 250 ml.

4.15 Blender - High speed, glass or stainless cup.

4.16 Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 in foil-covered glass container. Determine lauric-acid value (See Appendix I).

4.17 Silica gel - Davison code 950-08-08-226 (60/80 mesh).

4.18 Glass Wool - Hexane extracted.

4.19 Centrifuge Tubes - Pyrex calibrated (15 ml).

5. Reagents, Solvents and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.4 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.5 Sodium Sulfate - (ACS) Granular, anhydrous, conditioned for 4 hours @ 400 C.

5.6 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.7.1 Must contain 2% alcohol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.7.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.7.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.8 n-Hexane - Pesticide quality (NOT MIXED HEXANES).

5.9 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (Boiling range 30-60 C) - pesticide quality, redistill in glass if necessary.

5.10 Standards - Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016.

5.11 Anti-static Solution - STATNUL, Daystrom, Inc., Weston Instrument Division, Newark, N.J. 95212.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable when the response to dicapthon is at least 50% of full scale when ≤ .06 ng is injected for electron capture detection and ≤ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions, detector and column. Example chromatograms are shown in Figures 3 through 8 and provide reference operating conditions.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as a quality control check. When the routine occurrence of a pollution parameter is observed, quality control charts are also recommended (5).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equal to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For a sensitivity requirement of 1 μg/l, when using microcoulometric or electrolytic conductivity methods for detection take 100 ml of sample for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

8.3 Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 6-10 ml in the K-D evaporator on a hot water bath.

9.4 Qualitatively analyze the sample by gas chromatography with an electron capture detector. From the response obtained decide:

a. If there are any organochlorine pesticides present,

b. If there are any PCB's present,

c. If there is a combination of a and b,

d. If elemental sulfur is present,

e. If the response is too complex to determine a, b, or c.

f. If no response, concentrate to 1.0 ml or less, as required, according to EPA Method (4), pg. 28 and repeat the analysis looking for a, b, c, d, and e. Samples containing Aroclors with a low percentage of chlorine, eg. 1221 and 1232, may require this concentration in order to achieve the detection limit of 1 μg/l. Trace quantities of PCB's are often masked by background which usually occur in the samples.

9.5 If condition a exists, quantitatively determine the organochlorine pesticides according to (1).

9.6 If condition b exists, PCB's only are present, no further separation or cleanup is necessary. Quantitatively determine the PCB's according to 11. below.

9.7 If condition c exists, compare peaks obtained from the sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCB's from the organochlorine pesticides, continue as outlined in 10.4.

9.8 If condition d exists separate the sulfur from the sample using the method outlined in (10.3) followed by the method in (10.5).

9.9 If condition e exists then the following macro cleanup and separation procedures (10.2 and 10.3) should be employed and, if necessary, followed by the micro separation procedures (10.4 and 10.5).

10. Cleanup and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as, the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether cleanup is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to remove fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for the compounds of interest.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-35 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

10.2.4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition below). Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin
BHC
Chlordane
DDD
DDE
DDT
Heptachlor
Heptachlor Epoxide
Lindane
Methoxychlor
Mirex
Pentachloronitrobenzene
Strobane
Toxaphene
Trifluralin
PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

10.3.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

10.3.5 Analyze by gas chromatography.

10.4 Silica Gel Micro-Column Separation Procedure (7)

10.4.1 Activation for Silica Gel

10.4.1.1 Place about 20 gm of silica gel in a 100 ml beaker. Activate at 180 C for approximately 16 hours. Transfer the silica gel to a 100 ml glass stoppered bottle. When cool, cover with about 35 ml of 0.50% diethyl ether in benzene (volume:volume). Keep bottle well sealed. If silica gel collects on the ground glass surfaces, wash off with the above solvent before resealing. Always maintain an excess of the mixed solvent in bottle (approximately 1/2 in. above silica gel). Silica gel can be effectively stored in this manner for several days.

10.4.2 Preparation of the Chromatographic Column

10.4.2.1 Pack the lower 2 mm ID Section of the microcolumn with glass wool. Permanently mark the column 120 mm above the glass wool. Using a clean rubber bulb from a disposable pipet seal the lower end of the microcolumn. Fill the microcolumn with 0.50% ether in benzene (v:v) to the bottom of the 10/30 joint (Figure 1). Using a disposable capillary pipet, transfer several aliquots of the silica gel slurry into the microcolumn. After approximately 1 cm of silica gel collects in the bottom of the microcolumn, remove the rubber bulb seal, tap the column to insure that the silica gel settles uniformly. Carefully pack column until the silica gel reaches the 120 ± 2 mm mark. Be sure that there are no air bubbles in the column. Add about 10 mm of sodium sulfate to the top of the silica gel. Under low humidity conditions, the silica gel may coat the sides of the column and not settle properly. This can be minimized by wiping the outside of the column with an anti-static solution.

10.4.2.2 Deactivation of the Silica Gel

a. Fill the microcolumn to the base of the 10/30 joint with the 0.50% ether-benzene mixture, assemble reservoir (using spring clamps) and fill with approximately 15 ml of the 0.50% ether-benzene mixture. Attach the air pressure device (using spring clamps) and adjust the elution rate to approximately 1 ml/min. with the air pressure control. Release the air pressure and detach reservoir just as the last of the solvent enters the sodium sulfate. Fill the column with n-hexane (not mixed hexanes) to the base of the 10/30 fitting. Evaporate all residual benzene from the reservoir, assemble the reservoir section and fill with 5 ml of n-hexane. Apply

As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with 0.25 ml of n-hexane. Then assemble the upper section of the column. As the last of the n-hexane rinse reaches the surface of the sodium sulfate, add enough n-hexane (volume predetermined, see 10.4.3 below) to just elute all of the PCB's present in the sample. Apply air pressure and adjust until the flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see 10.4.3 below) in an accurately calibrated ampul. As the last of the n-hexane reaches the surface of the sodium sulfate, release the air pressure and change the collection ampul.

c. Fill the column with 0.50% diethyl ether in benzene, again apply air pressure and adjust flow to 1 ml/min. Collect the eluate until all of the organochlorine pesticides of interest have been eluted (volume predetermined, see 10.4.3 below).

d. Analyze the eluates by gas chromatography.

10.4.3 Determination of Elution Volumes

10.4.3.1 The elution volumes for the PCB's and the pesticides depend upon a number of factors which are difficult to control. These include variation in:

a. Mesh size of the silica gel

b. Adsorption properties of the silica gel

c. Polar contaminants present in the eluting solvent

d. Polar materials present in the sample and sample solvent

e. The dimensions of the microcolumns

Therefore, the optimum elution volume must be experimentally determined each time a factor is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and serially collect 1 ml elution volumes. Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for ether-benzene. Figure 2 shows the retention order of the various PCB components and of the pesticides. Using this information, prepare the mixtures required for calibration of the microcolumn.

10.4.3.2 In determining the volume of hexane required to elute the PCB's the sample volume (1 ml) and the volume of n-hexane used to rinse the column wall must be considered. Thus, if it is determined that a 10.0 ml elution volume is required to elute the PCB's, the volume of hexane to be added in addition to the sample volume but including the rinse volume should be 9.5 ml.

10.4.3.3 Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (9.4) indicates which Aroclors are present and provides the basis for selection of the ideal elution volume. This helps to minimize the quantity of organochlorine pesticides which will elute along with the low percent chlorine PCB's and insures the most efficient separations possible for accurate analysis.

10.4.3.4 For critical analysis where the PCB's and pesticides are not separated completely, the column should be accurately calibrated according to (10.4.3.1) to determine the percent of material of interest that elutes in each fraction. Then flush the column with an additional 15 ml of 0.50% ether in benzene followed by 5 ml of n-hexane and use this reconditioned column for the sample separation. Using this technique one can accurately predict the amount (%) of materials in each micro column fraction.

10.5 Micro Column Separation of Sulfur, PCB's, and Pesticides

10.5.1 See procedure for preparation and packing micro column in PCB analysis section (10.4.1 and 10.4.2).

10.5.2 Microcolumn Calibration

10.5.2.1 Calibrate the microcolumn for sulfur and PCB separation by collecting 1.0 ml fractions and analyzing them by gas chromatography to determine the following:

1) The fraction with the first eluting PCB's (those present in 1260),
2) The fraction with the last eluting PCB's (those present in 1221),
3) The elution volume for sulfur,
4) The elution volume for the pesticides of interest in the 0.50% ether-benzene fraction.

From these data determine the following:

1) The eluting volume containing only sulfur (Fraction I),
2) The eluting volume containing the last of the sulfur and the early eluting PCB's (Fraction II),
3) The eluting volume containing the remaining PCB's (Fraction III),
4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV).

10.5.3 Separation Procedure

10.5.3.1 Carefully concentrate the 6% eluate from the florisil column to 2.0 ml in the graduated ampul on a warm water bath.

10.5.3.2 Place 1.0 ml (50%) of the concentrate into the microcolumn with a 1 ml pipet. Be careful not to get any sulfur crystals into the pipet.

10.5.3.3 Collect Fractions I and II in calibrated centrifuge tubes.

Collect Fractions III and IV in calibrated ground glass stoppered ampules.

10.5.3.4 Sulfur Removal (9) - Add 1 to 2 drops of mercury to Fraction II stopper and place on a wrist-action shaker. A black precipitate indicates the presence of sulfur. After approxiately 20 minutes the mercury may become entirely reacted or deactivated by the precipitate. The sample should be quantitatively transferred to a clean centrifuge tube and additional mercury added. When crystals are present in the sample, three treatments may be necessary to remove all the sulfur. After all the sulfur has been removed from Fraction II (check using gas chromatography) combine Fractions II and III. Adjust the volume to 10 ml and analyze gas chromatography. Be sure no mercury is transferred to the combined Fractions II and III, since it can react with certain pesticides.

By combining Fractions II and III, if PCB's are present, it is possible to identify the Aroclor(s) present and a quantitative analysis can be performed accordingly. Fraction I can be discarded since it only contains the bulk of the sulfur. Analyze Fractions III and IV for the PCB's and pesticides. If DDT and its homologs, aldrin, heptachlor, or technical chlordane are present along with the PCB's, an additional micro-column separation can be performed which may help to further separate the PCB's from the pesticides (See 10.4).

11. Quantitative Determination

11.1 Measure the volume of n-hexane eluate, containing the PCB's and inject 1 to 5 μl into the gas chromatograph. If necessary, adjust the volume of the eluate to give linear response to the electron capture detector. The microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCB's.

11.2 Calculations

11.2.1 When a single Aroclor is present, compare quantitative Aroclor reference standards (e.g., 1242, 1260) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Microgram/liter} = \frac{[A]\ [B]\ [V_t]}{[(V_i)\ (V_s)]} \times [N]$$

$$A = \frac{\text{ng of Standard Injected}}{\Sigma \text{ of Standard Peak Areas}} = \frac{\text{ng}}{\text{mm}^2}$$

$B = \Sigma$ of Sample Peak Areas = (mm^2)

V_i = Volume of sample injected (μl)

V_t = Volume of Extract (μl) from which sample is injected into gas chromatograph

V_s = Volume of water sample extracted (ml)

N = 2 when micro column used
1 when micro column not used

Peak Area = Peak height (mm x Peak Width at
1/2 height

11.2.2 For complex situations, use the calibration method described below. Small variations in components between different Aroclor batches make it necessary to obtain samples of several specific Aroclors. These reference Aroclors can be obtained from Dr. Ronald Webb, Southest Environmental Research Laboratory, EPA, Athens, Georgia 30601. The procedure is as follows:

11.2.2.1 Using the OV-1 column, chromatograph a known quantity of each Aroclor reference standard. Also chromatograph a sample of p,p'-DDE. Suggested concentration of each standard is 0.1 ng/μl for the Aroclors and 0.02 ng/μl for the p,p'-DDE.

11.2.2.2 Determine the relative retention time (RRT) of each PCB peak in the resulting chromatograms using p,p'-DDE as 100. See Figures 3 through 5.

$$RRT = \frac{RT \times 100}{RT_{DDE}}$$

RRT = Relative Retention Time

RT = Retention time of peak of interest

RT_{DDE} = Retention time of p,p'-DDE

Retention time is measured as that distance in mm between the first appearance of the solvent peak and the maximum for the compound.

11.2.2.3 To calibrate the instrument for each PCB measure the area of each peak.

Area = Peak height (mm) x Peak width at 1/2 height. Using Tables 1 through 6 obtain the proper mean weight factor, then determine the response factor ng/mm^2.

$$ng/mm^2 = \frac{(ng_i)\ \frac{(\text{mean weight percent})}{100}}{(\text{Area})}$$

ng_i = ng of Aroclor Standard Injected

Mean weight percent = obtained from Tables 1 through 6.

11.2.2.4 Calculate the RRT value and the area for each PCB peak in the sample chromatogram. Compare the sample chromatogram to those obtained for each reference Aroclor standard. If it is apparent that the PCB peaks present are due to only one Aroclor then calculate the concentration of each PCB using the following formula:

$$ng\ PCB = ng/mm^2 \times Area$$

Where Area = Area (mm^2) of sample peak

ng/mm^2 = Response factor for that peak measured.

Then add the nanograms of PCB's present in the injection to get the total number of nanograms of PCB's present. Use the following formula to calculate the concentration of PCB's in the sample:

$$\text{Micrograms/Liter} = \frac{[ng]}{[V_s]}\ \frac{[V_t]}{[V_i]} \times [N]$$

V_s = volume of water extracted (ml)

V_t = volume of extract (μl)

Table 1

Composition of Aroclor 1221 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	31.8	15.8	1
14	19.3	9.1	1
16	10.1	9.7	2
19	2.8	9.7	2
21	20.8	9.3	2
28	5.4	13.9	2⎤ 85% 3⎦ 15%
32	1.4	30.1	2⎤ 10% 3⎦ 90%
⎡37 ⎣40	1.7	48.8	3 3
Total	93.3		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of seventeen results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

V_i = volume of sample injected (µl)

Σng = sum of all the PCB's in nanograms for that Aroclor identified

N = 2 when microcolumn used

N = 1 when microcolumn not used

The value can then be reported as Micrograms/ Liter PCB's reported as the Aroclor. For samples containing more than one Aroclor, use Figure 9 chromatogram divisional flow chart to assign a proper response factor to each peak and also identify the "most likely" Aroclors present. Calculate the ng of each PCB isomer present and sum them according to the divisional flow chart. Using the formula above, calculate the concentration of the various Aroclors present in the sample.

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

Table 2

Composition of Aroclor 1232 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
[20	17.8	2.4	2
21]			2
11	16.2	3.4	1
14	9.9	2.5	1
16	7.1	6.8	2
28	9.6	3.4	2] 40%
			3] 60%
32	3.9	4.7	3
37	6.8	2.5	3
40	6.4	2.7	3
47	4.2	4.1	4
54	3.4	3.4	3] 33%
			4] 67%
58	2.6	3.7	4
70	4.6	3.1	4] 90%
			5] 10%
78	1.7	7.5	4
Total	94.2		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of four results as a mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 3

Composition of Aroclor 1242 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	1.1	35.7	1
16	2.9	4.2	2
21	11.3	3.0	2
28	11.0	5.0	2 25% 3 75%
32	6.1	4.7	3
37	11.5	5.7	3
40	11.1	6.2	3
47	8.8	4.3	4
54	6.8	2.9	3 33% 4 67%
58	5.6	3.3	4
70	10.3	2.8	4 90% 5 10%
78	3.6	4.2	4
84	2.7	9.7	5
98	1.5	9.4	5
104	2.3	16.4	5
125	1.6	20.4	5 85% 6 15%
146	1.0	19.9	5 75% 6 25%
Total	98.5		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 4

Composition of Aroclor 1248 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
21	1.2	23.9	2
28	5.2	3.3	3
32	3.2	3.8	3
47	8.3	3.6	3
40	8.3	3.9	3] 85% 4] 15%
47	15.6	1.1	4
54	9.7	6.0	3] 10% 4] 90%
58	9.3	5.8	4
70	19.0	1.4	4] 80% 5] 20%
78	6.6	2.7	4
84	4.9	2.6	5
98	3.2	3.2	5
104	3.3	3.6	4] 10% 5] 90%
112	1.2	6.6	5
125	2.6	5.9	5] 90% 6] 10%
146	1.5	10.0	5] 85% 6] 15%
Total	103.1		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 5

Composition of Aroclor 1254 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
47	6.2	3.7	4
54	2.9	2.6	4
58	1.4	2.8	4
70	13.2	2.7	4] 25% 5] 75%
84	17.3	1.9	5
98	7.5	5.3	5
104	13.6	3.8	5
125	15.0	2.4	5] 70% 6] 30%
146	10.4	2.7	5] 30% 6] 70%
160	1.3	8.4	6
174	8.4	5.5	6
203	1.8	18.6	6
232	1.0	26.1	7
Total	100.0		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers are bracketed.

Table 6

Composition of Aroclor 1260 (B)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
70	2.7	6.3	5
84	4.7	1.6	5
98 104	3.8	3.5	[d] 5 60% 6 40%
117	3.3	6.7	6
125	12.3	3.3	5 15% 6 85%
146	14.1	3.6	6
160	4.9	2.2	6 50% 7 50%
174	12.4	2.7	6
203	9.3	4.0	6 10% 7 90%
232 244	9.8	3.4	[e] 6 10% 7 90%
280	11.0	2.4	7
332	4.2	5.0	7
372	4.0	8.6	8
448	.6	25.3	8
528	1.5	10.2	8
Total	98.6		

[a] Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of six results as a mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[d] Composition determined at the center of peak 104.

[e] Composition determined at the center of peak 232.

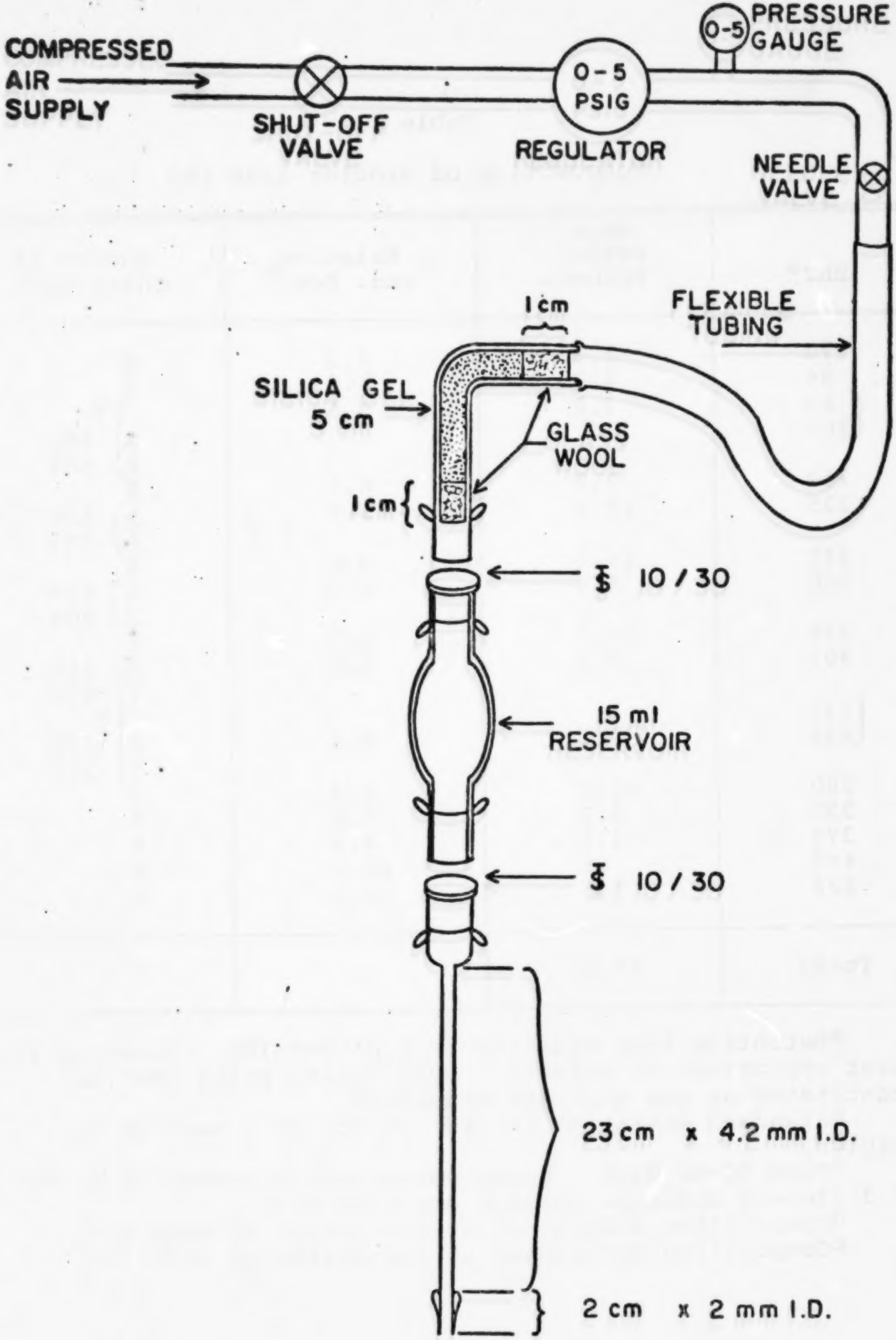

COMPRESSED
AIR
SUPPLY
SHUT-OFF
VALVE
0-5
PSIG
REGULATOR
0-5
PRESSURE
GAUGE
NEEDLE
VALVE
FLEXIBLE
TUBING
1 cm
SILICA GEL
5 cm
GLASS
WOOL
1 cm
$ 10 / 30
15 ml
RESERVOIR
$ 10 / 30
23 cm x 4.2 mm I.D.
2 cm x 2 mm I.D.

FIGURE 1. MICROCOLUMN SYSTEM

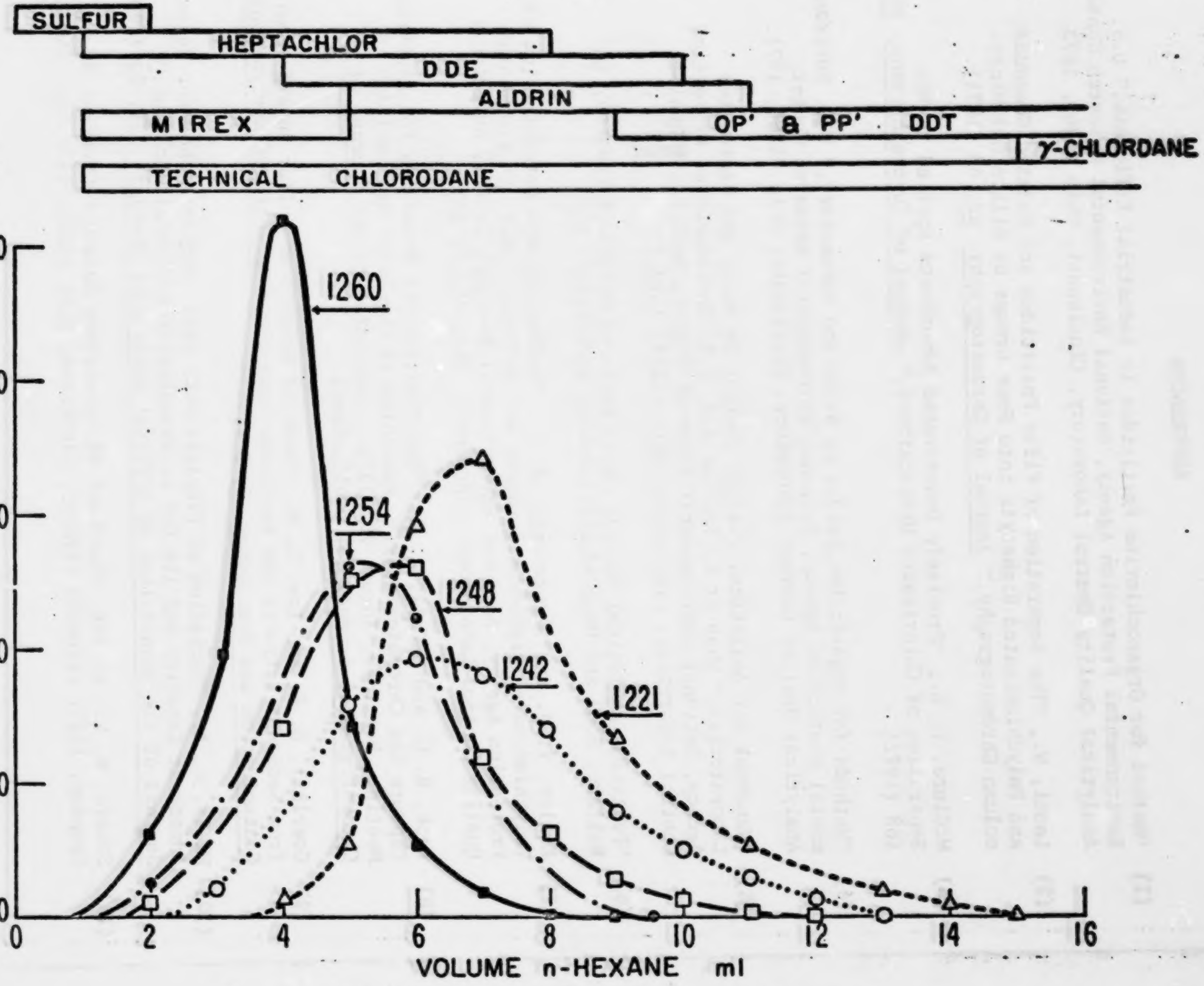


Figure 2. Aroclor Elution Patterns

REFERENCES

(1) "Method for Organochlorine Pesticides in Industrial Effluents," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(2) Leoni, V., "The Separation of Fifty Pesticides and Related Compounds and Polychlorinated Biphenyls into Four Groups by Silica Gel Micro-column Chromatography," Journal of Chromatography, 62, 63 (1971).

(3) McClure, V. E., "Precisely Deactivated Adsorbents Applied to the Separation of Chlorinated Hydrocarbons," Journal of Chromatography, 70, 168 (1972).

(4) "Methods for Organic Pesticides in Water and Wastewater," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(5) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(6) "Pesticide Analytical Manual," U.S. Dept. of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.

(7) Bellar, T. A. and Lichtenberg, J. J., "Method for the Determination of Polychlorinated Biphenyls in Water and Sediment," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(8) Webb, R. G. and McCall, A. C., "Quantitative PCB Standards for Electron Capture Gas Chromatography." Presented at the 164th National ACS Meeting, New York, August 29, 1972. (Submitted to the Journal of Chromatographic Science for publication).

(9) Goerlitz, D. F. and Law, L. M., "Note on Removal of Sulfur Interferences from Sediment Extracts for Pesticide Analysis," Bulletin of Environmental Contamination and Toxicology, 6, 9 (1971).

(10) Mills, P. A., "Variation of Florisil Activity: Sample Method for Measuring Adsorbent Capacity and its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).

(11) Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorptio of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil,= 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides:

Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

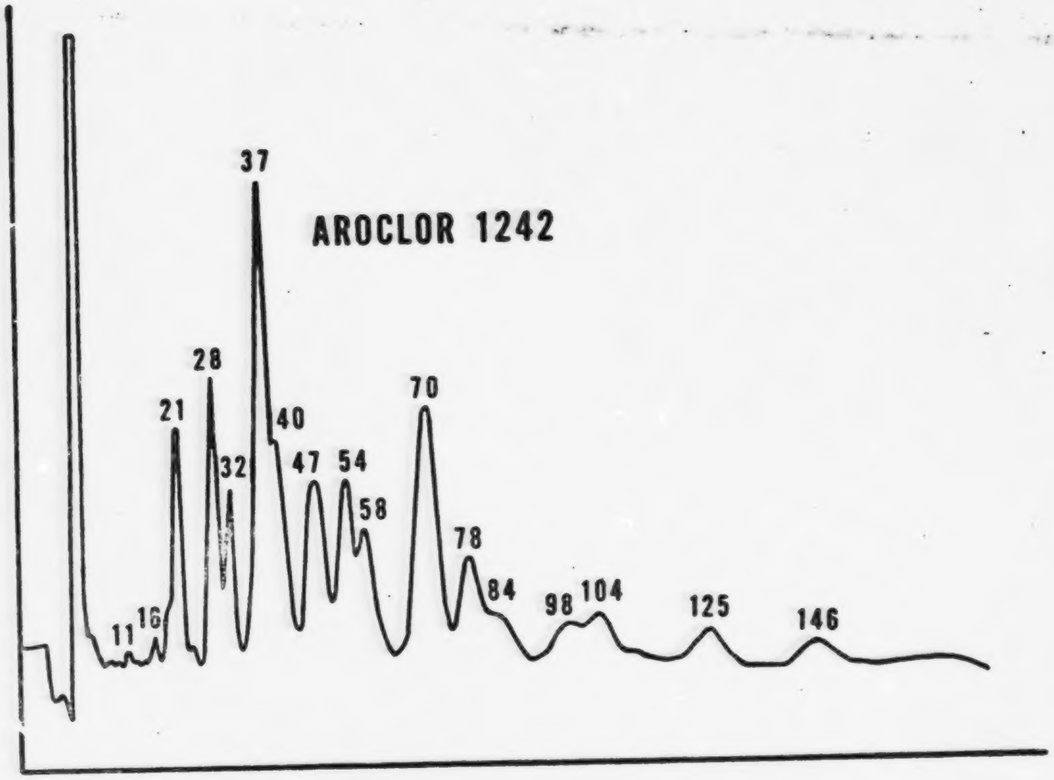


Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture

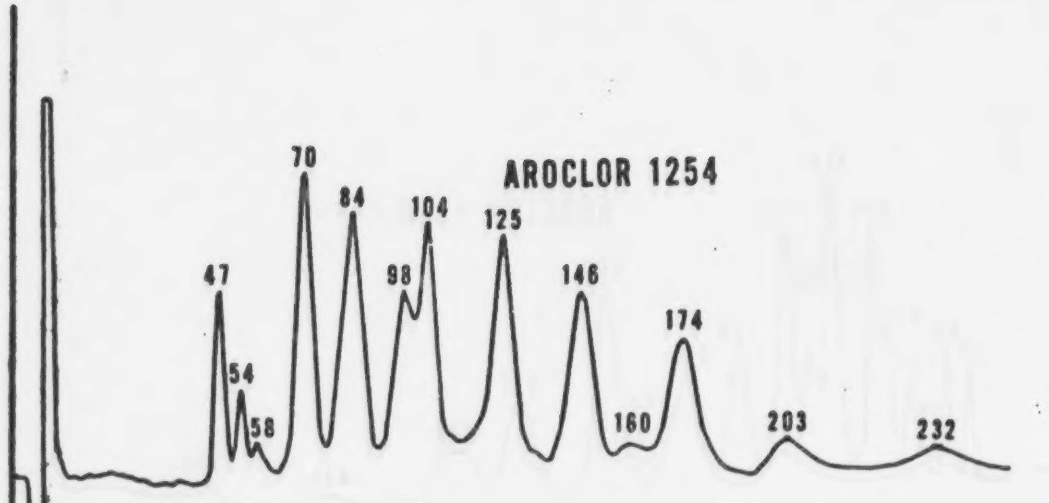


Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

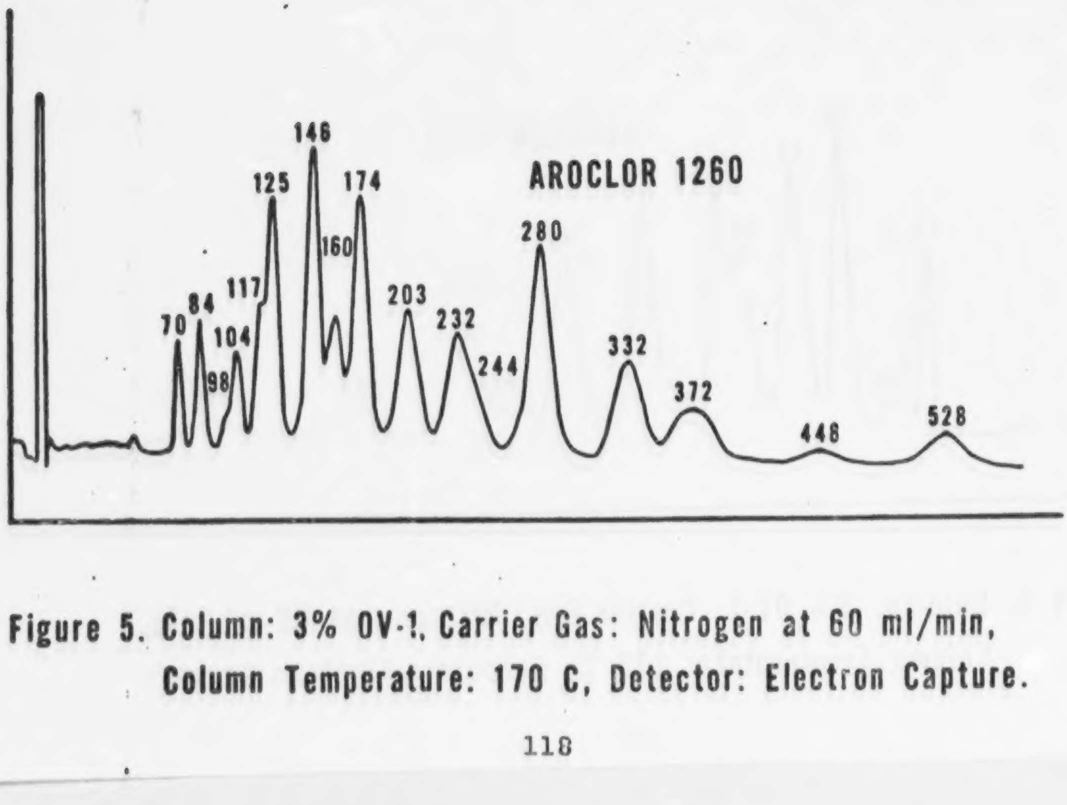


Figure 5. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

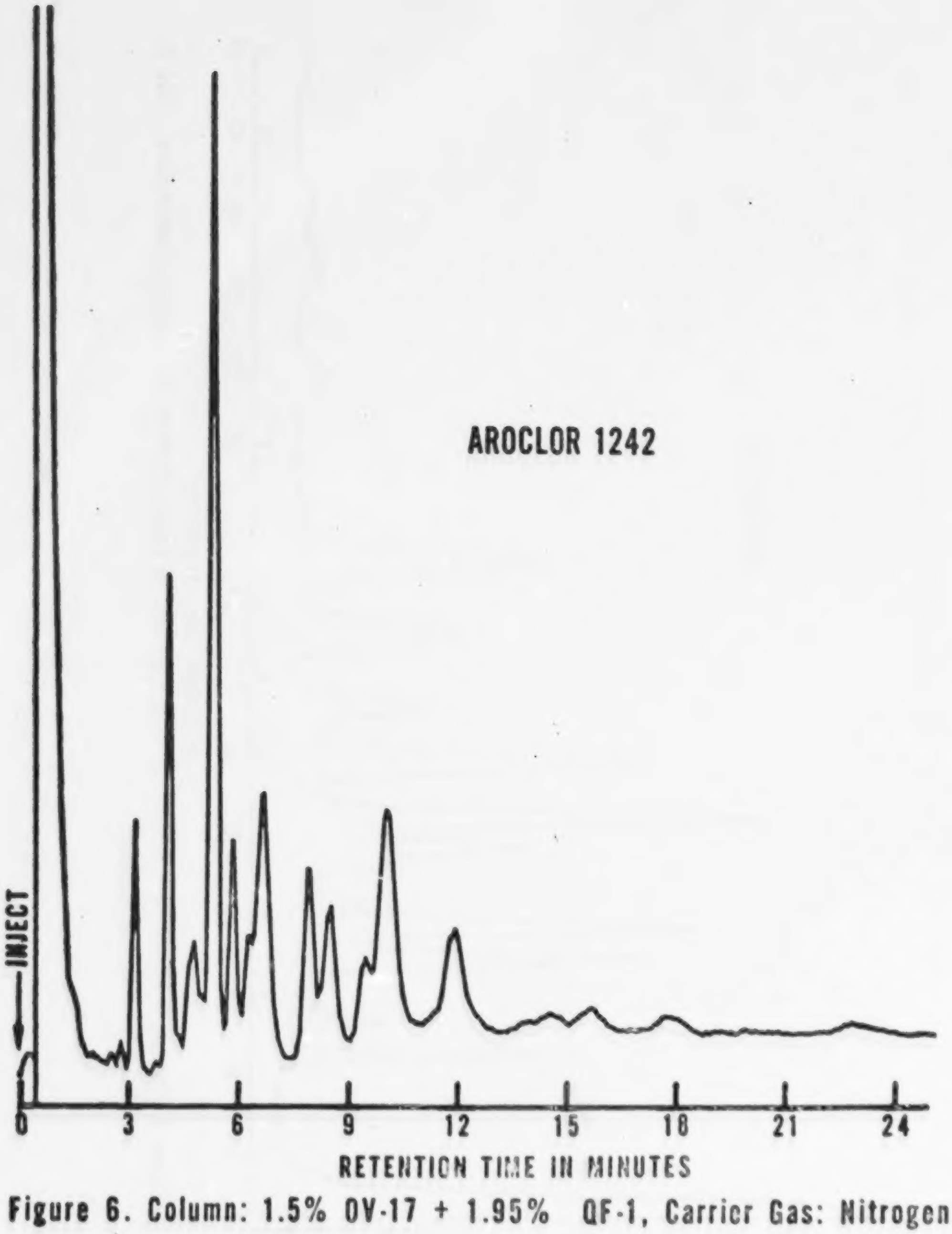


Figure 6. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

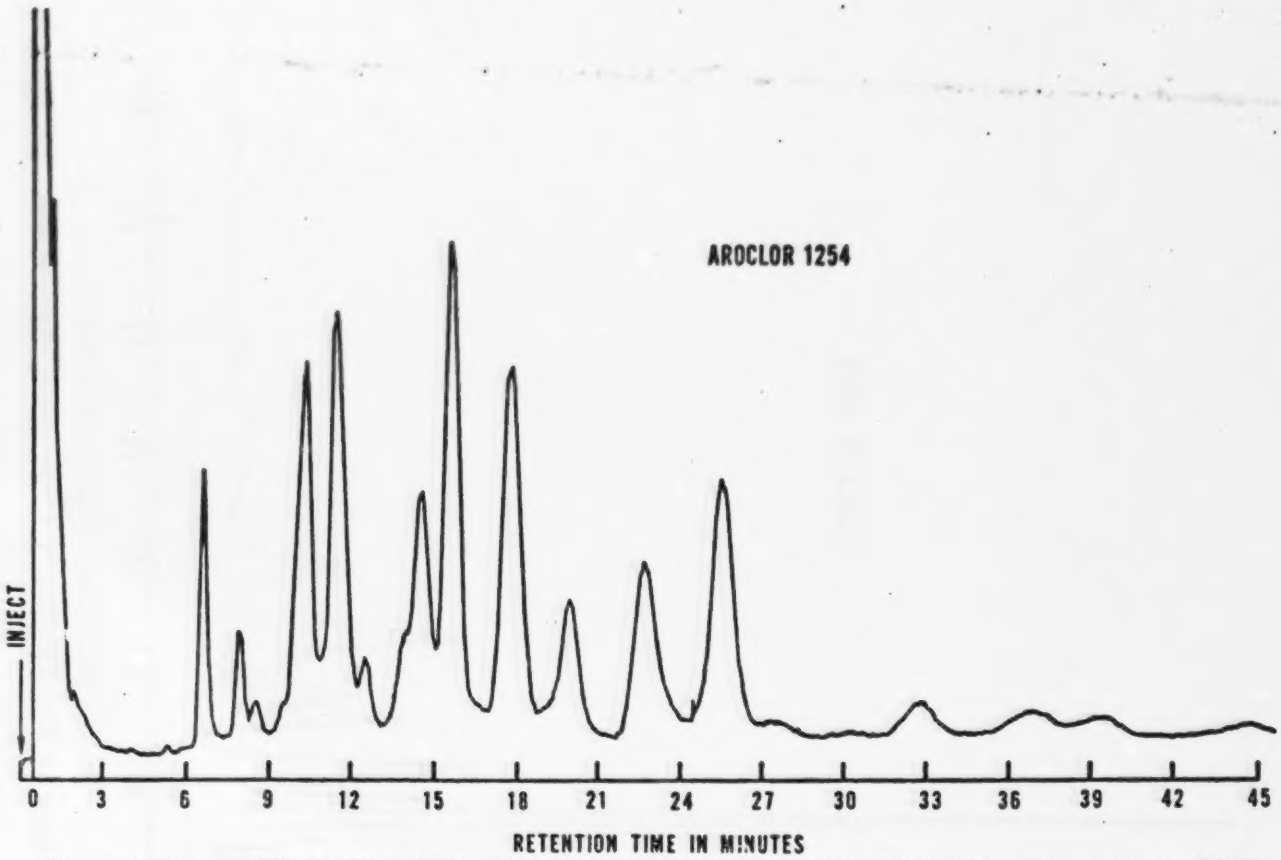


Figure 7. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

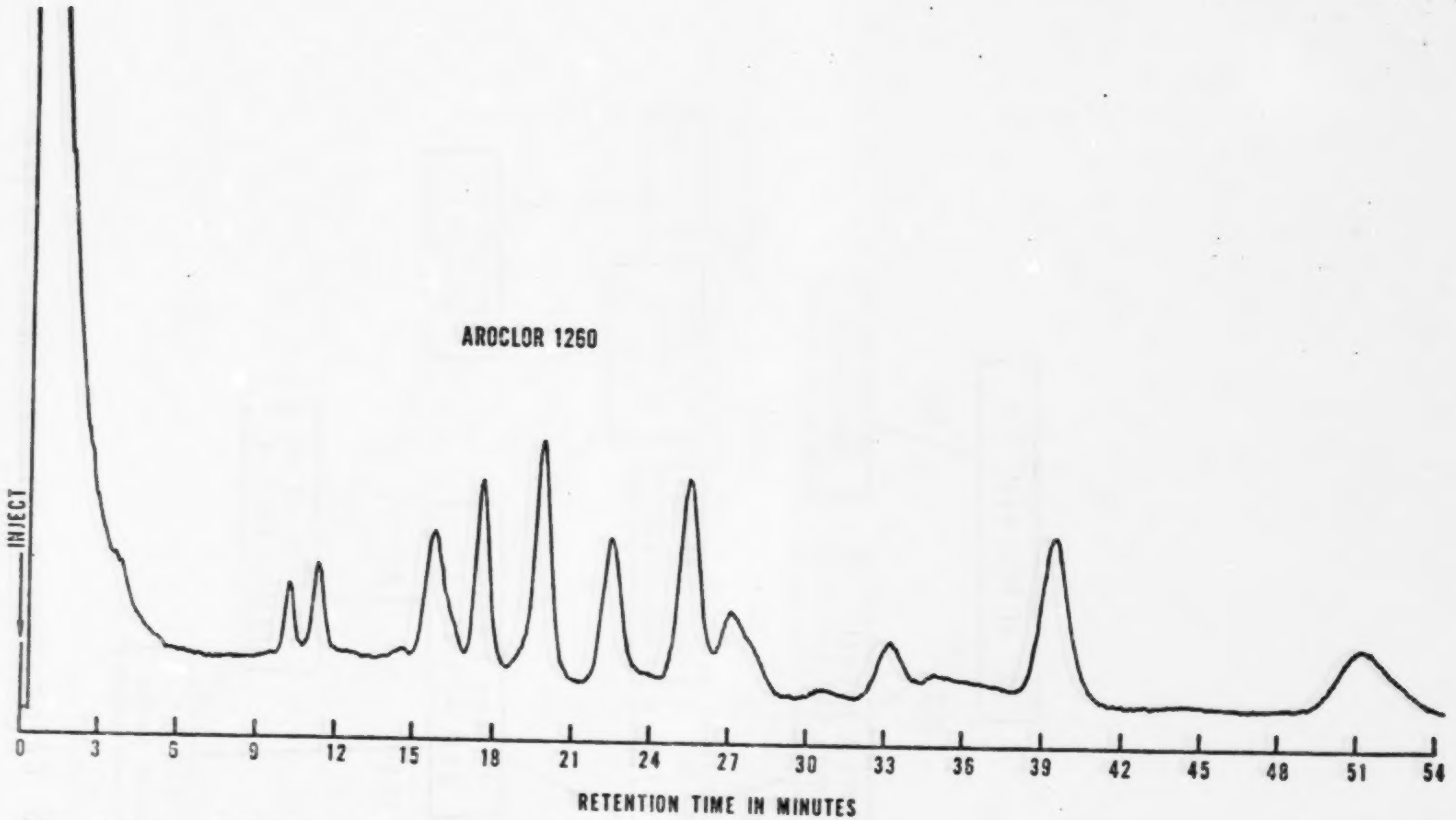


Figure 8. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200C, Detector: Electron Capture.

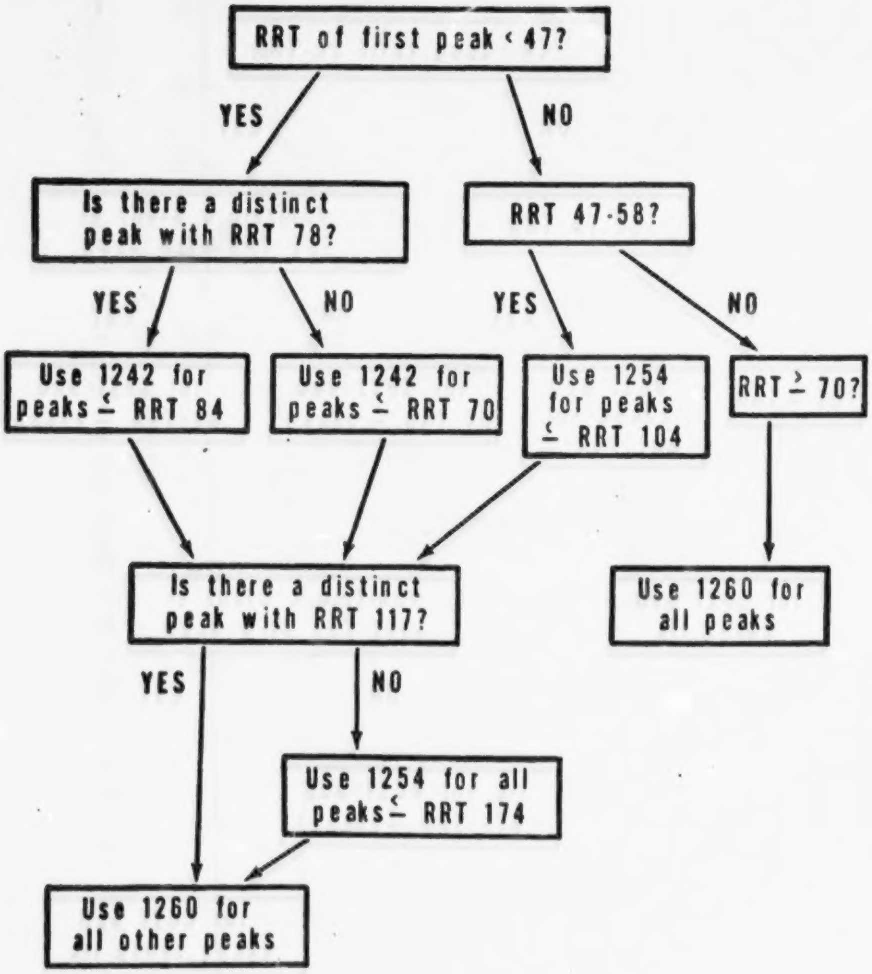

RRT of first peak < 47?
YES
NO
Is there a distinct peak with RRT 78?
RRT 47-58?
YES
NO
YES
NO
Use 1242 for peaks ≤ RRT 84
Use 1242 for peaks ≤ RRT 70
Use 1254 for peaks ≤ RRT 104
RRT ≥ 70?
Is there a distinct peak with RRT 117?
Use 1260 for all peaks
YES
NO
Use 1254 for all peaks ≤ RRT 174
Use 1260 for all other peaks

Figure 9. Chromatogram Division Flowchart (8).

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 758-486/1021

J. METHOD FOR ORGANOCHLORINE SOLVENTS IN THE ELUTRIATE TEST*

1. Scope and Application

1.1 This method describes a direct aqueous-injection (1)(2) procedure for the determination of gas chromatographable chlorinated hydrocarbons. The method is specific for hydrocarbons containing chlorine, iodine, and bromine. It is sensitive to approximately 1 mg/l. The compounds detected are composed of carbon, the above mentioned halogens and usually hydrogen; however, these compounds may also contain fluorine, oxygen, nitrogen, sulfur or phosphorus.

1.2 The method is useful only for organo-halide compounds with a water solubility exceeding 1 mg/l @ 22°C. Many commonly used organochlorine solvents meet this specification, i.e., carbon tetrachloride, 800 mg/l at 20°C, chloroform, 1000 mg/l at 15°C, methylene chloride, 2000 mg/l at 20°C (3).

2. Summary

2.1 If the sample is turbid it is initially centrifuged or filtered through a fiber glass filter in order to remove suspended matter. A three to ten microliter aliquot of the sample is injected into the gas chromatograph equipped with a halogen specific detector. The resulting chromatogram is used to identify and quantitate specific components in the sample. Results are reported in micrograms per liter. Confirmation of qualitative identifications are made using two or more dissimilar columns.

3. Sample Collection and Handling

3.1 A representative sample should be collected in a clean glass bottle. Containers such as a BOD bottle or a 1-quart wide mouth bottle with a TFE fluorocarbon cap liner should be used.

3.2 The sample volume should be in excess of 200 ml.

---

*Taken from "A Method for organochlorine solvents in industrial effluents" N.P.D.E.S. Appendex A, FED REG, 38, No. 75, Pt II, 1973.

3.3 The sample is best preserved by protecting it from phase separation. Since the majority of the chlorinated solvents are volatile and relatively insoluble in water, it is important that the sample bottle be filled completely to minimize air space over the sample. The sample must remain hermetically sealed up to the time it is analyzed. Refrigeration or freezing only encourages phase separation and should be avoided. Acidification will minimize the formation of non-volatile salts formed from chloroorganic acids and certain chlorophenols. However, it may interfere with the detection of acid degradable compounds such as chloroesters. Therefore, the sample history must be known before any chemical or physical preservation steps can be applied. To insure sample integrity, it is best to analyze the sample within 1 hour of collection.

4. Interferences

4.1 The use of a halogen specific detector eliminates any possibility of interference from compounds not containing chlorine, bromine, or iodine. Compounds containing bromine or iodine will interfere with the determination of organochlorine compounds. The use of two dissimilar chromatographic columns helps to minimize this interference and in addition this procedure helps to verify all qualitative identifications. When concentrations are sufficiently high, unequivocal identifications can be made using infrared or mass spectroscopy. Though non-specific, the flame ionization detector may be used for known systems where interferences are not a problem.

4.2 Ghosting is usually attributed to the history of the chromatographic system. Each time a sample is injected small amounts of various compounds are adsorbed on active sites in the inlet and at the head of the column. Subsequent injections of water tend to steam clean these sites resulting in non-representative peaks or displacement of the baseline. This phenomenom normally occurs when an analysis of a series of highly concentrated samples is followed by a low level analysis. The system should be checked for ghost peaks prior to each quantitative analysis by injecting distilled water in a manner identical to the sample analysis (5). If excessive ghosting occurs, the following maintenance should be applied, as required, in the order listed:

1) Multiple flushes with distilled water
2) Clean or replace the glass injector liner
3) Replace the chromatographic column

5. Apparatus and Materials

5.1 Gas Chromatograph - Equipped with programmed oven temperature controls and glass-lined injection port. The oven should be equipped with a column exit port and heated transfer line for convenient attachment to the halogen specific detector.

5.2 Detector Options:

5.2.1 Microcoulometric Titration

5.2.2 Electrolytic Conductivity

5.2.3 Flame Ionization

5.3 Recorder - Potentiometric strip chart recorder (10 in) compatible with the detector.

5.4 Syringes - 1 µl, 10 µl, and 50 µl.

5.5 BOD type bottle or 1 quart bottle with Teflon lined screw cap.

5.6 Volumetric Flasks - 500 ml, 1000 ml.

5.7 Syringe - Hypodermic Lur-lock type (30 ml).

5.8 Filter glass fiber filter - Type A (13 mm).

5.9 Filter holder - Swinny type hypodermic adapter (13 mm).

5.10 Glass stoppered ampuls - 10 ml

5.10.1 Non-Polar Column - 12 ft x 0.1 in ID x 0.125 in OD stainless steel column #304 packed with 5% OV-1 on chromosorb-W (60-80 mesh).

5.10.2 Moderately-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel column #304 packed with 5% carbowax 20 M on Chromosorb-W (60-80 mesh).

5.10.3 Highly-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with 5% 1,2,3-Tris-(2-cyanoethoxy) propane on Chromosorb-W (60-80 mesh).

5.10.4 Porous Polymer Column - 6 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with Chromosorb-101 (60-80 mesh).

6. Reagents

6.1 Chlorinated hydrocarbons reference standards

6.1.1 Prepare standard mixtures in volumetric flasks using contaminant free distilled water as solvent. Add a known amount of the chlorinated compounds with a microliter syringe. Calculate the concentration of each component as follows:

$$\text{mg/l} = (\text{Density of Compound})(\mu\text{l injected})\left\{\frac{1000}{\text{Dilution Volume (ml)}}\right\}$$

7. Quality Control

7.1 Duplicate quantitative analysis on dissimilar columns should be performed. The duplicate quantitative data should agree within experimental error (±6 percent). If not, analysis on a third dissimilar column should be performed. Spiked sample analyses should be routinely performed to insure the integrity of the method.

8. Selection Gas Chromatographic Column

8.1 No single column can efficiently resolve all chlorinated hydrocarbons. Therefore, a specific column must be selected to perform a given analysis. Columns providing only partially or non-resolved peaks are useful only for confirmatory identifications. If the qualitative nature of the sample is known then an efficient column selection can be made by reviewing literature (4). In doing this, one must remember that injection of large volumes of water can cause two serious problems not normally noted using common gas chromatographic techniques:

1) Water can cause early column failure due to liquid phase displacement.
2) Water passing through the column causes retention times and orders to change when compared to common sample solvent media, ie., hexane or air.

For these reasons column life and the separations obtained by direct aqueous injection may not be identical to those suggested in literature.

8.2 If nothing is known about the sample, a thermally stable non-polar column such as the OV-1 column is a good first choice. Temperature programming this column from room temperature to its upper limit will provide a wide molecular weight range analysis. Following this with a moderately polar column and a highly polar column provides efficient separations and corroborative identifications for a wide variety of common chlorinated solvents. The unique low molecular weight separations achieved on porous polymer columns is extremely useful when the samples contain a mixture of such compounds.

9. Sample Preparation

9.1 If the sample is turbid it should be filtered or contrifuged to prevent syringe plugging or excessive ghosting problems. Filtering the sample is accomplished by filling a 30 ml hypodermic syringe with sample and attaching the Swinny type hypodermic filter adaptor with a glass fiber filter "Type A" installed. Discard the first 5 ml of sample then collect the filtered sample in a glass stoppered ampule filled to the top. (One should occasionally analyze the non-filtered sample to insure that the filtering technique does not adversely effect the sample).

10. Method of Analysis

10.1 First, analyze the filtered sample of unknown composition by injection of a 3 to 10 µl into the gas chromatograph. The injection volume and detector sensitivity is recorded.

10.2 Prepare a standard mixture consisting of the same compounds in concentrations approximately equal to those detected in the sample. Chromatograph the standard mixture under conditions identical to the unknown.

11. Calculation of Results

11.1 Measure the area of each unknown peak and each reference standard peak as follows:

Area = [Peak Height][Width of Peak at 1/2 Height]

11.2 Calculate the concentration of each unknown as follows:

$$\text{mg/l} = \frac{(\text{Area of Sample peak})(\mu\text{l of Standard Injected})(\text{Conc'n of Standard})}{(\mu\text{l of Sample Injected})(\text{Area of Standard Peak})}$$

12. Reporting Results

12.1 Report results in mg/l. If a result is negative, report the minimum detectable limit, ie. <1 mg/l. When duplicate and spiked samples are analyzed, all data obtained should be reported.

References

1. "Tentative Recommended Practice for Measuring Volatile Organic Matter in Water by Aqueous - Injection Gas Chromatography", D2908-70T, 1971 Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis, American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.
2. Bellar, T. A. and Lichtenberg, J. J., "Method for the Determination of Chlorinated Organic Solvents by Direct Aqueous - Injection Gas Chromatography", U. S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268 (March 1973).
3. "Handbook of Chemistry and Physics", 48th Edition, The Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128. (1967-1968)
4. "Gas Chromatography Abstracts", Knapman, C.E.H., Editor, Institute of Petroleum, 61 New Cavendish Street, London W1M8AR, Annually 1958 to date, since 1970, also includes Liquid Chromatography Abstracts.
5. Dressman, R. C., "Elimination of Memory Peaks Encountered in Aqueous-Injection Gas Chromatography", Journal of Chromatographic Science, 8, 265 (1970).

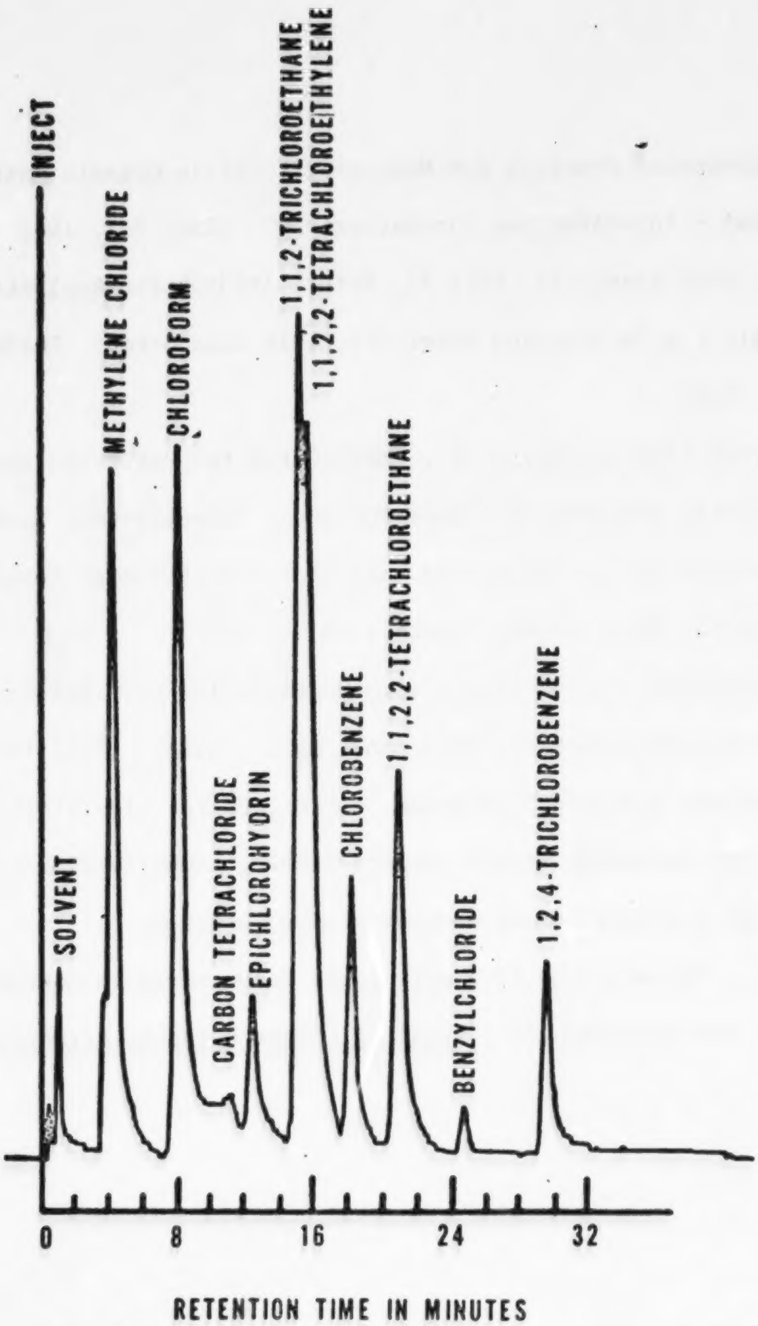


Figure 1. Column: Chromosorb-101, Temperature Program: 125 C for 4 min then 4 C/min up to 280 C., Carrier Gas: Nitrogen at 36 ml/min, Detector: Microcoulometric.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1152

VII. ANALYSES OF SEDIMENT

A. DRY SOLIDS

1. Scope and Application

1.1 This method is to determine the dry weight, wet weight ratio of the sediment.

1.2 All results of sediment analyses should be based on dry solids.

2. Summary of Method

2.1 A well mixed aliquot of a sediment sample is quantitatively transferred to a preweighed evaporating dish and evaporated to dryness at 103 - 105°C.

3. Apparatus

3.1 Evaporating dishes, porcelain.

4. Procedure

4.1 Heat the clean evaporating dish to 550 ± 50°C for 1 hour in a muffle furnace. Cool, desiccate, weigh and store in desiccator until ready for use.

4.2 Transfer an accurately weighed sediment of approximately 10 g to the preweighed dish.

4.3 Dry the sediment for 24 hours at 103 - 105°C. Cool in a desiccator and weigh.

4.4 Repeat the cycle of drying at 103 - 105°C, cooling, desiccating and weighing until a constant weight is obtained or until loss of weight is less than 4% of the previous weight.

5. Calculation

5.1 Calcualte dry solids

$$\text{Dry solids, g/kg} = \frac{(A-B) \times 1000}{\text{wt of wet sediment, g}}$$

A = wt in g of sample + dish

B = wt of dish in g

B. METALS IN BOTTOM SEDIMENTS BY ATOMIC ABSORPTION

1. Scope and Application

Metal complexes are freed by digestion of bottom sediment samples with concentrated HNO_3 acid and H_2O_2 (30%). This is not appropriate for mercury determination because of losses during sample digestion and ashing. Many other elements, such as Sn, Ti, W will not be appreciably extracted. Elements such as Cr, Al, Mn may be extracted, partially extracted or not measureably extracted, depending on their form in the sediment. The extraction of Cu, Cd, Zn, Pb and Ni will probably be nearly complete using this method. A wide range of elements can be determined by atomic absorption with the selection of the proper source (hollow cathode lamp).

1.1 Principle: The principle of atomic absorption and its application to the analysis of metals is based upon the element of interest being dissociated from its chemical bonds and placed into an unexcited, un-ionized "ground" state. It is then capable of absorbing at discrete lines of narrow band width radiation provided by a hollow cathode lamp with a cathode made of the element being sought. The absorption of the light by the metal in the ground state is related to the concentration of the metal being sought.

1.2 Interference: The atomic absorption method is relatively free of interferences.

2. Apparatus

2.1 Atomic absorption spectrophotometer.

2.2 Muffle furnace.

2.3 Pipettes.

2.4 250 ml beakers.

2.5 Watch glasses.

2.6 250 ml volumetric flasks.

2.7 Polyethylene bottles (60 ml).

2.8 Centrifuge (20,000 rpm).

3. Reagents

3.1 Nitric acid (conc. metal-free).

3.2 Hydrogen peroxide (30%).

3.3 Hydrochloric acid (conc. metal-free).

3.4 Ammonium chloride.

3.5 Calcium nitrate.

3.6 Glass redistilled water (metal-free).

3.7 Bottled acetylene gas.

3.8 Bottled nitrous oxide gas.

3.9 Compressed air.

4. Preparation of Standards

4.1 Prepare an appropriate stock standard from a commercial certified atomic absorption standard containing 1,000 mg/l of the metal. Standards may be prepared in the laboratory using pure metals.

4.2 Make up working standards to approximate the concentration in the sample using the dilute stock standard.

5. Preparation of Samples (except for mercury analysis)

5.1 Place 2.5 g of a well mixed bottom sediment sample in a 250 ml beaker, add 10 ml of conc. HNO_3 acid and 0.5 ml of H_2O_2 (30%).

5.2 Cover beaker with a watch glass and allow mixture to gently reflux for 2 hours on a hot plate.

5.3 Remove watch glass and evaporate to dryness. If the dry residue is a dark color, add a couple of drops of conc. HNO_3 and continue to heat. If the residue is still a dark color after repeating this process, proceed with the ashing.

5.4 Ash at 400-425°C for one hour in a muffle furnace and cool to room temperature.

5.5 Add 10 ml of acid mixture (200 ml of conc. HNO_3, 50 ml conc. HCl and 750 ml of redistilled water), 8 ml of 10% NH_4Cl and 0.4 ml of $Ca(NO_3)_2 \cdot 4H_2O$ (11.8 g/100 ml) to the ash.

5.6 Heat gently for 15 minutes and cool for five minutes or longer.

5.7 Transfer sample to a centrifuge tube, then rinse the digestion beaker with 10 ml redistilled water until a final volume of 30 ml. Centrifuge for 10 min. at 20,000 RPM.

5.8 Transfer the supernatant into a 100 ml volumetric flask.

5.9 Rinse the digestion beaker with 30 ml of redistilled water and transfer into the centrifuge tube. Centrifuge and add the supernatant to the previous mixture.

5.10 Rinse the centrifuge a third and final time.

5.11 Adjust the final volume to 100 ml with redistilled water. The mixture is ready for atomic absorption analysis.

6. Analytical Methods for Cu, Cd, Ni, Zn, Cr, Pb, Mn, Al and Other Metals

Follow procedures as outlined in manufacturer's manual.

7. Calculations

7.1 Wet Basis

By use of table from manufacturer's manual, convert percent absorption to absorbance.

$$\text{mg/kg} = \frac{\text{mg/l in std.}}{\text{absorbance of std.}} \times \text{dil. factor} \times \text{absorbance of sample} \times \frac{1{,}000}{\text{g of sample/l}}$$

7.2 Dry Basis

$$\text{mg/kg} = \frac{\text{mg/kg wet basis}}{\text{Dry solids, g/Kg}} \times 1000$$

Dry solids can be obtained from section V. A. 5.1

C. MERCURY (COLD VAPOR TECHNIQUE)*

1. Scope and Application

1.1 This procedure (1) measures total mercury (organic + inorganic) in soils, sediments, bottom deposits and sludge type materials.

1.2 The range of the method is 0.2 to 5 ug. The range may be extended above or below the normal range by increasing or decreasing sample size or through instrument and recorder control.

2. Summary of Method

2.1 A weighed portion of the sample is digested in aqua regia for 2 minutes at 70°C, followed by oxidation with potassium permanganate. Mercury in the digested sample is then measured by the conventional cold vapor technique (2,3).

3. Sample Handling and Preservation

3.1 Because of the extreme sensitivity of the analytical procedure and the omnipresence of mercury, care must be taken to avoid extraneous contamination. Sampling devices and sample containers should be ascertained to be free of mercury; the sample should not be exposed to any condition in the laboratory that may result in contact or air-borne mercury contamination.

4. Interferences

4.1 The same types of interferences that may occur in water samples are also possible with sediments, i.e., sulfides, high copper, high chlorides, etc. Substances causing excessive foaming will make the mercury stripping impossible.

---

*This is modified from a provisional method issued by Analytical Quality Control Laboratory, EPA, Cincinnati, Ohio.

4.2 A drop of octyl alcohol may help. Volatile materials which absorb at the 253.7 nm will cause a positive interference. In order to remove any interfering volatile materials, the dead air space in the BOD bottle should be purged before the addition of stannous sulfate.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer:* Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.

5.2 Mercury Hollow Cathod Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.3 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.4 Absorption Cell: Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells may be constructed from plexiglass tubing, 1" O.D. x 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter x 1/16" thickness) are cemented in place. Gas inlet and outlet ports (also of plexiglass but 1/4" O.E.) are attached approximately 1/2" from each end. The cell is strapped to a burner for support and aligned in the light beam to give the maximum transmittance.

---

*Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substitued for the atomic absorption spectrophotometer.

NOTE: Two 2" x 2" cards with one inch diameter holes may be placed over each end of the cell to assist in positioning the cell for maximum transmittance.

5.5 Air Pump: Any peristaltic pump capable of delivering 1 liter of air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfactory. (Regulated compressed air can be used in an open one-pass system).

5.6 Flowmeter: Capable of measuring an air flow of 1 liter per minute.

5.7 Aeration Tubing: Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return. Straight glass tubing terminating in a coarse porous frit is used for sparging air into the sample.

5.8 Drying Tube: 6" x 3/4" diameter tube containing 20 grams of magnesium perchlorate (see Note 2). The apparatus is assembled as shown in the accompanying diagram.

NOTE 2: In place of the magnesium perchlorate drying tube, a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Aqua Regia: Prepare immediately before use by carefully adding three volumes of conc. HCl to one volume of conc. HNO_3.

6.2 Sulfuric Acid, 0.5N: Dilute 14.0 ml of conc. sulfuric acid to 1.0 liter.

6.3 Stannous Sulfate: Add 25 g stannous sulfate to 250 ml of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use.

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution:

Dissolve 12 grams of sodium chloride and 12 grams of hydroxylamine sulfate in distilled water and dilute to 100 ml. Check for mercury. Any mercury present can be removed by sparging the solution with nitrogen.

NOTE 3: A 10% solution of stannous chloride may be substitued for(6.3) and hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate in 6.4.

6.5 Potassium Permanganate: 5% solution, w/v. Dissolve 5 grams of potassium permanganate in 100 ml of distilled water.

6.6 Stock Mercury Solution: Dissolve 0.1354 grams of mercuric chloride in 75 ml of distilled water. Add 10 ml of concentrated nitric acid and adjust the volume to 100.0 ml. 1 ml = 1 mg Hg.

6.7 Working Mercury Solution: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug per ml. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7. Calibration

7.1 Transfer 0, 0.5, 1.0, 2.0, 5.0 and 10 ml aliquots of the working mercury solution containing 0 to 1.0 ug of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 10 ml. Add 5 ml of aqua regia and heat for 10 minutes in a water bath at 70°C. Allow the sample to cool then add 50 ml distilled water and 15 ml of $KMnO_4$ solution to each bottle and return to the water bath for 1.5 hours. Cool and add 6 ml of sodium chloride - hydroxylamine sulfate solution to reduce the excess permanganate. Add 50 ml of distilled water. Treating each bottle individually, add 5 ml of stannous sulfate solution and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value (see Note 3). Close the bypass valve, remove the fritted tubing from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

NOTE 3: Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 N $KMnO_4$ and 10% H_2SO_4

b) 0.25% iodine in a 3% KI solution

A specially treated charcoal that will absorb mercury vapor is also available from Barnebey and Cheney, E. 8th Ave., and North Cassidy St., Columbus, Ohio 43219, Cat. #580-13 or #580-22.

8. Procedure

8.1 Weigh triplicate 0.2 g portions of sample and place each portion in the bottom of a BOD bottle. Add 5 ml of distilled water and 5 ml of aqua regia. Heat for 10 minutes in a water bath at 70°C. Cool, add 50 ml distilled water and 15 ml potassium permanganate solution to each sample bottle. Stopper the bottle, mix sample thoroughly and place in the water bath or oven at 70°C for 1.5 hours. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate. Add 55 ml of distilled water. Treating each bottle individually, add 5 ml of stannous sulfate and immediately attach the bottle to the aeration apparatus. Continue as described under 7.1.

8.2 An alternate digestion procedure employing an autoclave may also be used. In this method 5 ml of conc. H_2SO_4 and 2 ml of conc. HNO_3 are added to the 0.2 grams of sample. 5 ml of saturate $KMnO_4$ solution is added and the bottle covered with a piece of aluminum foil. The samples are autoclaved at 121°C and 15 lbs. for 15 minutes. Cool, make up to a volume of 100 ml with distilled water and add 6 ml of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Purge the dead air space and continue as described under (7.1).

9. Calculation

9.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

9.2 Calculate the mercury concentration in the sample by the formula:

$$\text{ug Hg/gm} = \frac{\text{ug Hg in the aliquot}}{\text{wt. of the aliquot in gms.}}$$

Report the mean value of the triplicate aliquots.

9.3 Report mercury concentrations as follows:

Below 0.1 ug/gm, < 0.1; between 0.1 and 1 ug/gm, to the nearest 0.01 ug; between 1 and 10 ug/gm, to nearest 0.1 ug; above 10 ug/gm, to nearest ug.

10. Precision and Accuracy

The following standard deviations on replicate sediment samples were recorded at the indicated levels; 0.29 ug/g ± 0.02 and 0.82 ug/g ± 0.03. Recovery of mercury at these levels, added as methyl mercuric chloride, was 97 and 94% respectively.

11. References

11.1 Bishop, J.N., "Mercury in Sediments", Ontario Water Resources Comm., Toronto, Ontario, Canada, 1971.

11.2 Hatch, W. R., and Ott, W. L., "Determination of Sub-Microgram Quantities of Mercury by Atomic Absorption Spectrophotometry", Anal. Chem. 40, 2085 (1968).

11.3 Bradenberger, H. and Bader, H., "The Determination of Nanogram Levels of Mercury in Solution by a Flamless Atomic Absorption Technique", Atomic Absorption Newsletter, 6, 101 (1967).

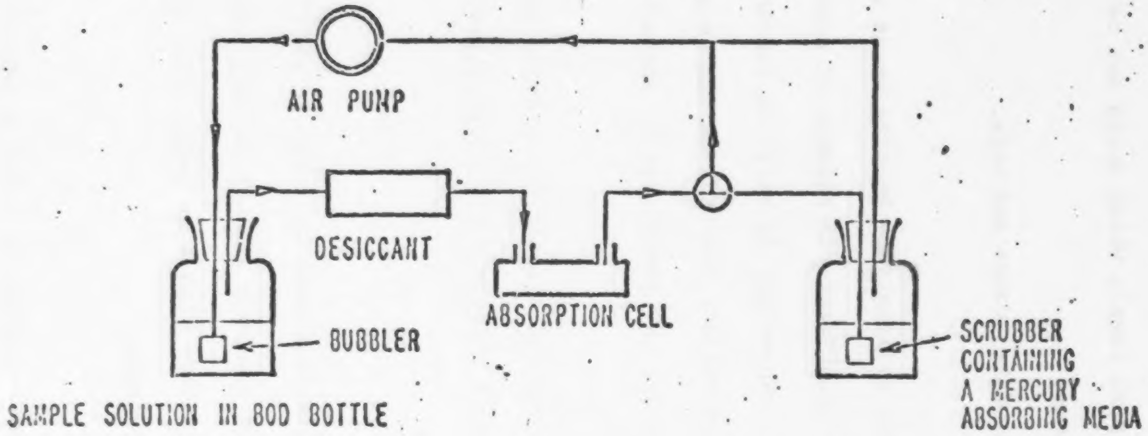

AIR PUMP
DESICCANT
ABSORPTION CELL
BUBBLER
SAMPLE SOLUTION IN BOD BOTTLE
SCRUBBER CONTAINING A MERCURY ABSORBING MEDIA

FIGURE 1. APPARATUS FOR FLAMELESS MERCURY DETERMINATION.

D. OIL AND GREASE

1. Scope and Application

1.1 This method measures the hexane extractable material from an acidified sediment sample which would not be volatilized during the procedure.

1.2 It includes soaps, fats, waxes and oils.

2. Summary of Method

2.1 Drying of acidified sediments by heating leads to low results. Magnesium sulfate monohydrate is capable of combining 75 per cent of its own weight in water in forming the heptahydrate. Magnesium sulfate monohydrate can be used to dry sludge. After drying, the grease can be extracted with hexane.

3. Interference

3.1 Elemental sulfur; certain organic dyes; oxidation of extract and loss in weight of residue due to volatilization of low boiling components.

4. Sampling and Storage

4.1 Every possible precaution must be taken to obtain a representative sample. When analyses can not be made immediately, samples may be preserved with 1 ml conc. H_2SO_4 for each 80 g of sample, or by freezing.

5. Apparatus

5.1 Extraction apparatus, soxhlet or A.S.T.M. apparatus.

6. Reagents

6.1 Hydrochloric acid conc.

6.2 Magnesium sulfate monohydrate: Prepare Mg $SO_4 \cdot H_2O$ by drying overnight a thin layer of Mg $SO_4 \cdot 7H_2O$ in an oven at 103°C.

6.3 Hexane, boiling point 69°C.

6.4 Grease free cotton: Nonabsorbent cotton after extraction with hexane.

7. Procedure

7.1 In a 250 ml beaker weigh a 20 g sample of wet sludge, of which the dry-solids content is known.

7.2 Acidify to a pH 2.0 (generally 0.3 ml conc. HCl is sufficient).

7.3 Add sufficient Magnesium sulfate, monohydrate, to remove the moisture. It should be added in small portions with stirring until a dry coarse powder is formed.

7.4 Remove the solids and grind in a porcelain mortar.

7.5 Add the powder to a paper extraction thimble. Wipe the beaker and mortar with small pieces of filter paper moistened with hexane and add to the thimble. Fill the thimble with small glass beads.

7.6 Extract in a soxhlet apparatus using hexane at a rate of 20 cycles per hour for 4 hours.

7.7 If any turbidity or suspended matter is present in the extraction flask, remove by filtering through grease-free cotton into another weighed flask. Rinse flask and cotton with hexane.

7.8 Distill hexane from the extraction flask in water at 70°C. Dry by placing on a steam bath and drawing air through the flask with a vacuum for 15 minutes. Cool in a desiccator for 30 minutes and weigh.

7.9 If a large analytical balance with a precision of ± 0.1 mg is unavailable to weigh the extraction flask, the following alternative method could be used. Evaporate the hexane from the extraction flask in water at 70°C to approximately 5 ml. Transfer the residue to a preweighed 50 ml beaker, rinse the flask with 5 ml hexane and combine the rinse with the residue. Repeat the rinsing two more times. Dry the combined residue and rinses by placing on a 70°C water bath, cool in a desiccator and weigh. Repeat drying process to a constant weight of ± 0.5 mg.

8. Calculation

8.1 Wet Basis

$$\text{mg/kg} = \frac{\text{mg of residue}}{\text{grams of sample}} \times 1000$$

8.2 Dry Basis

$$\text{mg/kg} = \frac{\text{mg/kg wet basis}}{\text{Dry solids in g/kg}} \times 1000$$

Dry solids can be obtained from section V. A. 5.1.

9. Reference

Standard Methods for the Examination of Water and Wastewater, 12th Ed., APHA, Inc., N.Y. 1965, 531-532.